THESIS

IDENTIFYING RISK FACTORS FOR EARLY PREGNANCY LOSS IN HOLSTEIN COWS

Submitted by

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ABSTRACT

IDENTIFYING RISK FACTORS FOR EARLY PREGNANCY LOSS IN HOLSTEIN DAIRY COWS

Adequate reproductive performance is a key factor in the success of dairy production. However, events such as early pregnancy loss occur in 15 to 17% of dairy pregnancies and result in diminished fertility and increased culling within herds. Identifying risk factors for pregnancy loss will help farmers use evidence to formulate effective breeding and management protocols to maximize efficiency and welfare. This thesis is focused on identifying risk factors in early pregnancy and explaining the impacts that factors such as body condition and health status pose on the maintenance of pregnancy.

Chapter 1 presented relevant literature involving the transition period, reproductive advancements, and reproductive challenges within the dairy industry.

The objective of chapter 2 was to characterize the associations between body condition score (BCS) and BCS change, utilizing an automated camera system during early lactation and close to artificial insemination, and pregnancy loss. A secondary objective was to identify the impact of disease on pregnancy loss over multiple periods throughout lactation. Overall, the dynamics of BCS differed between animals that lost pregnancy and those that maintained pregnancy. During the period close to artificial insemination, low BCS, and a significant loss in BCS, as well as disease resulted in higher rates of pregnancy loss.

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CHAPTER 1: LITERATURE REVIEW

Transition Period

In dairy cows, the transition period can be defined as the 3 weeks before to 3 weeks after parturition (Grummer et al., 1995; Drackley, 1999). During this period, the highest number of infectious diseases and metabolic disorders can occur due to nutritional, physiological, and social changes (Goff and Horst, 1997). The shift from advanced gestation into the lactating stage plays a key role in the genesis of these negative outcomes. Animals experience lactogenesis, the onset of milk secretion, which induces a negative energy balance and calcium deprivation (Drackley, 1999). The increase of negative health events is worsened by the immunosuppression experienced due to the sudden shift of energy and nutrients. The net energy and metabolizable protein consumed by the animals during this period is far under the requirement needed for mammary use and needed to supply maintenance needs (Bell, 1995). Among the most common peripartal diseases associated with this negative energy balance are milk fever, ketosis, retained fetal membranes, metritis, and displaced abomasum (Drackley, 1999; Eposito et al., 2014, Contreras and Sordillo, 2011).

Despite health outcomes being the primary focus of treatment and the largest association with pregnancy loss, metabolic imbalances due to negative energy balance often occurs before immunosuppression. Altered lipid metabolism during the transition period is a central component of the resulting compromised cow status. Briefly, non-esterified fatty acids and ketone bodies are utilized as alternate energy sources by the mammary gland, in response to the lowered glucose and insulin levels within the blood (Drackley, 1999). Fat is mobilized in adipose tissue and moved to the liver, muscle, and other tissues (Roberts et al., 1981). Excessive lipid mobilization can lead to periparturient health problems as the quantity of ketone bodies produced in a short period of time can build up. The sudden changes cows experience can lead to fatty livers and ketosis (Drackley, 1999).

Cow behavior is also modified throughout the transition period. Drackley, reported a decline in feed intake beginning 3 weeks before calving, followed by slow increases in intake following calving (Drackley, 1999; Osborne et al., 2002). Additionally, rest times for dairy cows can shift during this period as they are not able to attain the same level of comfort. Disturbed rest time can shift plasma cortisol and heart rates, ultimately increasing the susceptibility to disease (Ladewig and Smidt, 1989; Huzzey et al., 2005).

In addition to physiological and feeding behavior changes, animals experience new social dynamics. Social dynamics can lead to a higher amount of competition between animals which can lead to displacement (Olofsson, 1999; Huzzey et al., 2006). Studies comparing competition in multiparous cows and primiparous cows found that competition at the feed bunk could alter behavior to increase standing or waiting time, leading to an increased risk of lameness and disease during the transition period (Greenough and Vermunt, 1991; Singh et al., 1993; Proudfoot et al., 2009).

Dairy cow parity plays an important role in determining feeding and nutritional needs based on the quantity of production. Overall, multiparous cows typically will produce more milk and are larger animals than primiparous cows (Beauchemin and Rode, 1994). There have been limited studies regarding behavioral differences in parity of dairy cow. It is important to consider that in transition cows especially, due to differences in body weight and milk production there are additional differences in parity behavior when it comes to laying, social, and feeding behavior (Neave at al., 2017).

It has been found that acute changes in animal behavior throughout the transition period can be predictive of the performance and future health of the animal (Stevenson et al., 2020). To mitigate the negative effects of negative energy balance, farmers focus their efforts on improving the adjustment to the transition period, including nutritional and environmental factors that can be managed (Singh, 2019). Managing the transition period must additionally account for risk factors, environmental factors, milk yield, nutrition, and social dynamics (LeBlanc, 2010; Chapinal et al., 2012).

Negative energy balance

As productive efficiency has increased milk yield per cow, high-yielding cows will require a greater portion of absorbed nutrients to the mammary tissue for milk production, and hence require a greater nutrient intake. This cause-and-effect relationship between high milk production and increasing nutrient needs results in animals voluntarily consuming greater quantities of feed to support milk production (Bauman et al., 1985; Reynolds et al., 2004). Through selective breeding, the energy usage of dairy cows has been altered from approximately 69% of energy for maintenance and 31% for milk production in 1944, to 35% for maintenance and 65% for milk production in 2016 (Bauman et al., 1985; VandeHaar and St-Pierre, 2006; Gerber et al., 2011; Baumgard et al., 2017). Increasing milk yield leads to a rise in physiologic demands to regulate the mammary glands and milk synthesis through hemeostasis and hemeorhesis (Baumgard et al., 2017).

The process of hemeostasis in dairy cows, was first explained as the regulation and coordination of tissues and organs to maintain equilibrium (Cannon, 1929). The primary role or hemeostasis is the maintenance on glucose control, primarily through insulin and glucagon to assure adequate hepatic and muscle glycogen storage (Bauman and Elliot, 1983; Vernon and

Saski, 1991). The second form of regulation, hemeorhesis, results in the physiological process to regulate pregnancy and lactation (Bauman and Currie, 1980; Bauman, 2000). Through the process of hemeorhesis, the animal will change responsiveness and sensitivity of tissues to support increasing lactation (Bell and Bauman, 1997).

In dairy cows, negative energy balance results from nutrients transferred from the cow to use for her own demands, to the fetus or mammary tissue through hemeorhesis (LeBlanc, 2010; Contreras and Sordillo, 2011). Hemeorhesis is exacerbated by high milk production as it demands the mobilization of more lipids, resulting in the lack of utilizing glucose efficiently (insulin resistance), hypoglycemia, and hyperinsulinemia (Contreras and Sordillo, 2011). Since cows experience insulin resistance, reduced feed intake, negative energy balance, resulting in reduced immune system and animals can be susceptible to bacterial contamination of the uterus, it is not surprising that the incidence of disease is prominent (LeBlanc, 2010). A result of a greater negative energy balance during the transition period, ovarian cyclicity could be delayed as non-esterified fatty acid concentrations can lead to a decreased GnRH and LH production (Miqueo et al., 2019). Additionally, anestrus is correlated with negative health events including dystocia, displaced abomasum, ketosis, retention of fetal membranes and endometritis (Walsh et al., 2007; Vieira-Neto et al., 2014).

Although it is possible to mobilize non-esterified fatty acids and create energy through oxidation in the Krebs cycle, this puts a considerable strain on the liver and as fatty acids accumulate within the liver, ketone bodies are produced and released into blood circulation as an alternative energy source (Drackley, 1999; LeBlanc, 2010; Horst et al., 2018). The process of ketone bodies being produced in high numbers and released into circulation is termed ketosis and is often treated with Dextrose and propylene glycol (Bashir et al., 2016). Although ketosis can be subclinical and result in lameness or ruminal disorders, it can be widely recognized by clinical signs including a decreased dry matter intake resulting in a loss of body condition score, hypoglycemia, lowered milk production, and behavior changes (Bashir et al., 2016). The concern of ketosis emphasizes the importance of maintaining proper nutrition and minimizing the negative energy balance throughout the transition period. Studies have found animals will have more successful health outcomes when managing the demands of the transition period by doubling energy and protein requirements, including vitamin and antioxidants supply (vitamin A, B, and E, selenium, beta-carotene) and minerals (calcium and vitamin D, magnesium, phosphorus dietary cation diets) (Reddy et al., 2016; Gilbert, 2016). Nutritional physiology during the transition period is impacted by many factors and without proper management can result in changes of immune function and events mediated by the immune system (Overton and Waldron, 2004).

Immune Function

Within the transition period, the normal function of the immune system is altered due to cortisol concentrations activated by the hypothalamic-pituitary-adrenal axis. The change in immune function results in reduced cytokines, chemoattractants, platelet activating factors, complement proteins, antimicrobial peptides, markers for adhesion between cells, and cell migration into tissues, ultimately reducing cellular function and lactogenesis (Martin et al., 2016; Stoop et al., 2016; Bassel and Caswell, 2018; Meglia et al., 2018; Alhussien and Dang, 2019). Since cellular immune function is reduced, negative health events including mastitis or metritis, are difficult for the animal to fight off and can lead to worsened illness (Trevisi and Minuti, 2018).

Leukocytes are one of the predominant immune modulating cells that utilize fatty acids as energy. Specifically, the fatty acid palmitate has the ability to increase leukocyte function by altering structure which results in an increase of proinflammatory cytokines of leukocyte origin (Contreras and Sordillo, 2011; Agrawal et al., 2017). When infections occur, leukocytes participate in anaerobic glycolysis which unlike oxidative phosphorylation, can harm the animal (Horst et al., 2018). The process of anaerobic glycolysis in an animal with excess non-esterified fatty acids, can lead to further negative effects including a reduction of DNA synthesis, cytokine and antibody production, antigen presentation, chemotaxis and diapedesis (Conteras and Sordillo, 2011; LeBlanc, 2010; Ingvartsen and Moyes, 2013; 2015). To summarize, non-esterified fatty acid concentration can alter leukocyte functionality and although it can increase function, with increasing severity and duration, will result in a reduction of immune system function.

Reproductive Efficiency

Artificial Insemination (AI)

Through selective breeding, the dairy industry as a whole has become much more efficient in breeding for economically relevant traits. Following sustained reductions in fertility, in the last 20 years, conception rates at 21 days have risen from 14% to 50% in high producing Holstein herds (Carvalho et al., 2018).

It is estimated the length of estrus in dairy cows can vary from 6 to 30 hours with a mean of 17 total, 19.3 for cows and 16.1 for heifers (Hammond, 1927). It has been reported that spermatozoa survive for only 24 hours in the cervix and subsequently uterus, leading to a lowered conception (Andreev, 1937). Spermatozoa can survive in the cervix, but when exposed to the vagina the reproductive cells did not survive long (Beschlebnov, 1938). Since spermatozoa do not survive long, the incorporation of frozen-thawed semen and embryos became an important part of dairy progression (Polge and Rowson, 1952; Wilmut and Rowson, 1973). The utilization of frozen-thawed semen and embryos allowed for AI and embryo transfer as a way to improve genetics and breeding practices (Vishwanath, 2003; Hasler, 2014).

A side effect of the evolution in dairy production has been a decline in herd reproductive efficiency, which has been improved with the selection of reproductive traits (Washburn et al., 2002; Berglund, 2008). As animals have been bred for a higher efficiency of milk production, it is important to discuss the quality of semen for bulls. Bulls are often maintained on collection schedules that produce high quantities of sperm when compared to historic numbers (Hahn et al., 1969; Saacke and White et al., 1972; Amann et al., 1974). Even with concentrated sires producing vast amounts of sperm, it is surprising to find that although inbreeding exists in the population, inbreeding among sires had no effect on semen quality (Tseveenjav et al., 2018).

Donor sires have specific requirements to be identified and devoid of disease. Sires can be tested annually for disease and sperm quality following the Certified Semen Services division of the National Association of Animal Breeders. Each dose of preserved sperm will contain 20x10^6 total spermatozoa (Viswanath, 2003). Although it has often been implicated that genetics increasing milk yield in dairy cows leads to decreased fertility, often inadequate body condition and management result in decreased fertility, including conception, pregnancy loss, and anestrus cows (Bello et al., 2012; Carvalho et al., 2014; Zachut and Moallem, 2017; Weigel, 2006). The changes in environment and management, often have a large negative impact on dairy cattle fertility (Lucy, 2001). The increasing rates of early embryonic mortality are less impacted by the sperm quality or function and more related to environmental or management factors (Satori et al., 2002).

Historically, heritability for reproduction depict slow rates of progress or improvement (Nadaraja et al., 1988; Stålhammar et al., 1994). Determining genetic evaluations for fertility have limitations in hypersensitivity (VanRaden and Tooker, 2003). Genetic evaluation for heritability indicates possibilities to select for both production and reproduction, yet these traits are antagonistic (VanRaden et al., 2002). Since it is known that there is an unfaborable genetic correlation between milk production and reproduction, it is essential to balance fertility and calving performance alongside milk production. It has been shown that some dairy breeds show the potential to maintain good reproductive abilities, without the loss in milk production which would lead to more sustainable breeding programs (Berglund, 2008).

Reproductive techniques have evolved to incorporate in vitro embryo produced animals, ovum pick up for oocyte recovery, and in vitro fertilization (Pieterse et al., 1988; Looney et al., 1994). In vitro embryo production uses the concepts of embryo transfer and ovum pick up to produce large scale collection and breeding of superior genetics (Hasler, 2014). Additionally, cloning techniques have become incorporated through the process of somatic cell nuclear transfer which utilizes the genetics and morphology of a known animal (Cibelli et al., 1998). Through this process, the dairy industry has been able to edit morphological features such as polled traits and health benefits such as mastitis resistance (Carlson et al., 2016; Liu et al., 2014). For the dairy industry, the most beneficial advancement in breeding has been the use of sexed semen, a process that only recently has been possible to produce live sperm. The accuracy rate for X or Y chromosome sperm can be up to 90%, but often the process of sexing can result in cellular damage. With cellular damage to sperm, a decrease in conception rate and embryo production is possible (DeJarnette et al., 2009; Mikkola and Taponen, 2017).

Ovulation Synchronization

The timing of breeding is an essential part of successful conception and pregnancy (Ferguson and Skidmore, 2013). Historically, understanding signs of estrus is critical to timely breeding without an ovulation synchronization protocol. Often “vaginal secretions” is recognized as a sign leading into estrus and during estrus (Brown 1944; Hammond, 1927). Ovulation occurs 24 to 48 hours after the beginning of estrus (Hammond, 1927). Since ovulation occurs in a short window with signs of estrus, it is imperative that estrus behavior is accurately noted for breeding.

Often, it presents an impediment to herd fertility when animals either fail to express estrus or handlers fail to detect it. It was estimated that 28.5% of cows in estrus failed to be detected, so technology has assisted in monitoring behavior in both pasture and free stall systems (Kamphuis et al., 2012; Lopez et al., 2005). Estrus can be difficult to detect in anovulatory cows or animals with insufficient plasma estradiol that do not express normal estrus response, and high producing animals have shorter durations of estrus and lessened behavior responses including standing events and times as well as lower plasma estradiol concentrations and multiple ovulations (Lopez et al., 2005).

Hofstad (1941) performed a study specifically looking at early breeding. He found when cows were bred before the sixtieth day following parturition, abortions, metritis, dystocia and retained placenta rates were high while conception rate was low. He found the opposite to be true when breeding after sixty days, leading to a generalized understanding to wait until after the first sixty days after calving to breed. Researchers have also suggested breeding two times in a single estrus if the first insemination was early in the period (Andreev, 1937). When receiving multiple insemination services, the majority conceived at the first and second service, but some animals took five or more services to conceive (Trimberger and Davis, 1945). Overall, season or breed have not been found to significantly differ the length of estrus and ovulation time. Additionally, one study noted animals bred more than 6 hours but less than 24 hours before ovulation had a more successful conception and those bred after ovulation were given poor results of conception (Trimburger, 1948).

Multiple timed artificial insemination protocols, primarily the Ovsynch protocol, have been in use, so animals could be inseminated without estrus detection (Purseley et al., 1995; Schmitt et al., 1996). As an example, the use of the Ovsynch protocol has allowed for more timely breeding and a predictable day of ovulation (Moreira et al., 2000). Double-Ovsynch or G6G protocols additionally improve the precision of AI service rate and increase the rate of pregnancy per AI (Santos et al, 2017). Timed artificial insemination uses an injection of gonadotropin-releasing hormone during the estrus cycle, causing ovulation and synchronizes follicular waves (Thatcher et al., 1989; Macmillan and

Thatcher, 1991).

Diagnosis of Pregnancy

When diagnosing a cow as pregnant, the original method was transrectal palpation of the uterus 35 to 40d after insemination (Stevenson and Britt, 2017). Depending on the operation, 67% of all operations performed pregnancy diagnosis monthly, while larger operations greater than 500 cows performed pregnancy exams every two weeks or even weekly, while smaller operations tended to perform exams monthly. In 2014, the most routine methods of pregnancy confirmation were transrectal palpation pregnancy 85.7%, transrectal ultrasonography 27.4% and blood test to identify glycoprotein levels associated with pregnancy 4.1% (USDA, 2016). Transrectal ultrasonography has been a growing pregnancy tool as it is able to detect pregnancy at 20 days, instead of the 35-40 days needed for rectal palpation (Fricke et al., 2016). Unfortunately, ultrasonography requires training for proper use and can result in false-positive results when fluid associated with estrus is noted (Stevenson and Britt, 2017; Fricke, 2002). The last method of pregnancy detection by obtaining blood samples, does so by measuring pregnancy-associated glycoproteins in the blood (Ricci et al., 2015). Blood concentration differences are detectable after 25 days of pregnancy, yet these concentrations will rise and fall in early pregnancy, so it was suggested to conduct a pregnancy diagnosis at a peak in pregnancy-associated glycoproteins, close to 32 days after AI (Ricci et al., 2015). Both serum diagnosis of pregnancy as well as ultrasonography, allow early pregnancy diagnosis, yet can also result in a false positive or false negative. However, the knowledge of early pregnancy provides insight into possible early pregnancy loss that otherwise would be unknown if animals were not tested before day 45 as 10-15% of embryos are lost in early pregnancy (Santos et al., 2004).

Reproductive Challenges

Anovulation

Resuming normal cyclicity postpartum is an essential aspect of dairy cow breeding and herd profitability (Dubuc et al., 2012). Delayed ovarian cyclicity, also known as anovulation, is estimated to affect 20% (15-50%) of dairy cows evaluated at 60 days in milk (Cerri et al., 2004; Walsh et al., 2007; Santos et al., 2009; Monteiro et al., 2021). First, postpartum ovulation occurs on average by 30 days in milk, yet there is a large variation in the timing of ovulation (McCoy et al., 2006; Walsh et al., 2007; Galvão et al., 2010). Anovulation is often diagnosed by the absence of the corpus luteum and insufficient status of circulating progesterone or by transrectal palpation or ultrasonography for ovarian cysts (Opsomer et al., 2000; Santos et al., 2016; Monteiro et al., 2021).

Negative energy balance in early postpartum has a significant impact on the interval resumption of ovulation (Beam and Butler, 1998). The presence of elevated concentrations of non-esterified fatty acids in serum, led to animals remaining anovular by 49 days post-partum (Ribeiro et al., 2013). In addition to non-esterified fatty acids, calcium homeostasis has a direct impact on ovulation and animals with hypocalcemia have both delayed estrus and lowered pregnancy rates (Ribeiro et al., 2013; Martinez et al., 2012). Hypocalcemia can lead to metritis and endometritis, and in cases animals were found to have low serum Ca concentrations when they had uterine diseases (Ribeiro et al., 2013). Hypocalcemia leads to impaired leukocyte activation and reduced neutrophil function, relating to further diseases (Hammon et al., 2006; Martinez et al., 2012). The homeostasis of calcium levels is essential to immune cell function which consequently when not maintained, can lead to increased susceptibility to disease and decreased fertility (Ribeiro et al., 2013).

The risk factors for anovulation include the duration of the dry period, milk production, incidence of disease, and body condition score (Opsomer et al., 2000; Gümen et al., 2003; Lopez et al., 2005; Santos et al., 2009; Ribeiro et al., 2013). Dry period has a positive correlation with increased risk of anovulation, while milk production has a negative association or no association (Opsomer et al., 2000; Gümen et al., 2003; Santos et al., 2009). It is thought that the bacteria and products of inflammation in the uterus have a direct influence on follicular development which will in turn compromise ovulation (Sheldon et al., 2009). In addition, clinical and subclinical diseases as well as body condition greatly increase the chance of anovulation (Opsomer et al., 2000; Ribeiro et al., 2013; Caixeta et al., 2017). In a series of studies, it was found that with a greater loss in body condition post calving or a BCS of less than 2.75, animals had a delayed ovulation after postpartum (Opsomer et al., 2000; Lopez et al., 2005; Santos et al., 2009; Crowe et al., 2015;). A recent study found that reproductive diseases, hyperketonemia, digestive problems, mastitis, respiratory diseases and lameness were greater in anovular cows (Monteiro et al., 2021). The same study additionally demonstrated a low BCS at 35 days in milk and multiple diseases were strong risk factors for anovulation by measuring the size of follicles (Monteiro et al., 2021).

Anovulation can impact future pregnancy as by 50-60 days in milk, anovulation is associated with a decreased pregnancy rate for several months (Walsh et al., 2007; Santos et al., 2009). Animals that resumed cycling early (<21 days in milk), had a greater success in conception and pregnancy than those occurring after 21d (Opsomer et al., 2000; Galvão et al., 2010). The status of animals as anovulatory ultimately leads to decreased reproductive performance which in turn will increase the chance of culling (Dubuc et al., 2012). It was found that the incidence of pregnancy loss between 30-53 days gestation was greatest in anovular cows when compared to intermediate cyclic cows in diestrus and cyclic cows induced to ovulate (Bisinotto et al., 2010). Additionally, there are changes within the conceptus cells in anovular cows (Ribeiro et al., 2016). When there are changes to the genetic sequence of conceptus cells, there is an increased likelihood of the maternal immune system destroying those cells, resulting in pregnancy loss (Ribeiro et al., 2016). Studies suggest reproductive management should include health and nutrition programs to reduce periparturient problems, which in turn will reduce the prevalence of anovulation before breeding. Additionally, identifying anovular animals and increasing progesterone by using timed breeding techniques may help future fertility (Santos et al., 2016).

Pregnancy at first Artificial Insemination (AI1)

During the peripartum period, animals are in a negative energy balance which leads to a decrease in dry matter intake and further, body condition score (Roche et al., 2009). Postpartum health is compromised, resulting in a negative impact on performance of dairy cows and further negative health outcomes such as uterine, metabolic and other health disorders, all of which are risk factors for decreased reproductive abilities (Santos et al., 2010; Ribeiro et al., 2016; Carvalho et al., 2019). Although there have been advancements in breeding and timed AI programs, pregnancy at first AI in anovular cows is still poor (McDougall, 2010; Ribeiro et al. 2011; 2012). Studies have found that one of the reasons anovular cows have problems maintaining pregnancy is due to low levels of circulating progesterone, and when increasing these levels pregnancy increased from 35% to 48% (Larson et al., 2007).

It was reported that there is a positive association between pregnancy and increased body condition, and reduced body condition loss in early lactation (Roche et al., 2009). Factors such as excessive loss in BCS and postpartum health problems are associated with an extended anovulation, reduced pregnancy at AI and pregnancy loss (Santos et al., 2009; 2010). Ribeiro et al., summarized that minimizing health problems, loss of BCS and anovulation is essential in achieving reproductive efficiency (Ribeiro et al., 2013). Assessment of body condition predicted the outcome of pregnancy at first AI and increasing the monitoring of body condition can provide insight onto when to stop inseminating animals that fail to conceive (Inchaisri et al., 2012; Pinedo et al., 2022). Pinedo et al., found an association between successful pregnancy at AI1 and body condition and change in body condition, they discovered reductions in body condition closer to AI1 resulted in lower odds of pregnancy (Pinedo et al., 2022).

Body condition can be an indicator of health and therefore can be a helpful tool in monitoring nutritional state and reproductive success (Robeiro et al., 2013; Heuer et al., 1999). Thus, by observing body condition and disease variables, it is possible to predict effects on fertility and subsequent reproduction.

Pregnancy Loss

Pregnancy loss can have varying definitions for each study, but generally is defined as pregnancy and the failure to reconfirm a previously diagnosed pregnancy (McDougall et al., 2005). The only true definitive markers of pregnancy failure remain to be reductions in fluid volume and fetus size, loss of heartbeat, and floating debris within placental fluids (Ealy and Seekford, 2019). It is, however, additionally possible to use pregnancy detection tools such as ultrasonography and serum analysis to identify pregnancy failures through measurements of crown-rump length, reduction in pregnancy associated glycoproteins, alterations in circulating miRNA, reducing interferon stimulating genes and reduced circulation of progesterone (P4) (Ealy and Seekford, 2019).

Advances in reproduction has improved conception rates to approximately 80 to 90% of artificial insemination at timely ovulation, yet still subsequent pregnancies fail to reach term in 45 to 65% of lactating dairy cows (Santos et al., 2004; Wiltbank et al., 2016). The Committee on Bovine Reproductive Nomenclature (1972) defined the period of 42 days of gestation as the embryo period and after 42 days of gestation to birth as the fetal period. Pregnancy loss in dairy cows can occur throughout several periods of gestation with differing causes. The first period of pregnancy loss occurs during the first week after breeding, resulting from a failure of conception, which therefore did not result in a true pregnancy (Maillo et al., 2015). Only 50-60% of fertilized zygotes will be viable and develop to the proper stage by day 5 (Pohler et al., 2015; Wiltbank et al., 2016). During this period, pregnancy loss can be impacted by environment and hormonal conditions. Hormonal conditions can stem from improper P4 levels, and environmental impacts can result from heat stress and feed nutrient concentrations, among others (Ryan et al.,1993; Hackbart et al., 2010). The second period occurs from days 8 to 27, encompassing embryo elongation and maternal recognition of the pregnancy. In this period, 20 to 40% of pregnancies will fail (Pohler et al., 2016; Wijma et al., 2016; Wiltbank et al., 2016). The embryo must signal its presence to the maternal system to maintain the corpus luteum (CL) in order to preserve the pregnancy (Wiltbank et al., 2016). The circulation of P4 is again essential to maintain pregnancy and suboptimal P4 can lead to detrimental effects on the embryo development (Forde et al, 2011; Bridges et al., 2013). The third period occurs from days 28-60, resulting from defects or delays in development of the cholorioallantioic placentomes or embryos resulting in corpus luteum regression or embryo death (Pinedo et al., 2020). In this period of time, further losses occur at rates ranging from 6 to 39% (Chebel et al., 2004; Moore et al., 2005; Hernandez et al., 2012). During this period, risk factors can include anovulation at the end of the voluntary waiting period, reduced P4 circulation, farm protocols, change in body condition score, uterine diseases and non-uterine diseases (Wiltbank et al., 2016). The fourth period occurs during the third month of pregnancy, resulting from animals carrying twins in the same uterine horn. This period has significantly lowered risks as it is more related to placentome growth and the necessary nutrients as well as crowding (Wiltbank et al., 2016).

The size of the corpus luteum was once considered as a predictor of pregnancy, which was discovered to be inaccurate. Rather the size of the peri-ovulatory follicle can predict pregnancy failure because of reduced estradiol and P4 production (Starbuck et al., 2004; Perry et al., 2005; López-Gatius et al., 2012). In lactating dairy cattle, large follicles can be predictive of pregnancy failure between 30- and 60-days gestation (Pereira et al., 2016). The highest risk of pregnancy loss is in the first 60 days of gestation and decreases after this period of early gestation (Ealy and Seekford, 2019). Most of pregnancy loss occurs as embryo loss and after 50 days into gestation pregnancy loss is characterized as fetal death (Santos et al., 2004). Most of these pregnancy losses occur prior to the period of maintenance of the corpus luteum, high producing animals continue to have high sources of pregnancy loss up to 42-56 days after artificial insemination. Early pregnancy loss (EPL) can be estimated to 35-45% of pregnancy loss while late pregnancy loss is estimated to be less than 10% (Lucy, 2001; Stevenson, 2001). EPL has been an overwhelmingly high source of pregnancy loss since the 1950s and 1960s. Utilizing ultrasonography (US), pregnancy can be diagnosed at 24-26 days of gestation. Lowered concentration of P4, twin ovulation, body condition, age and service sire can all play roles in the maintenance of pregnancy during this early period.

Long-term and Economic Impacts of Pregnancy Loss

There have been limited studies regarding the long-term impacts of pregnancy loss, but those published have noted pregnancy loss to be associated with an 80 day increase from first service to conception, and an extension of calving interval by 77 days (Fourichon et al., 2000). Pregnancy loss can additionally result in endometritis, in cases up to 23.2% of pregnancy losses resulted in endometritis (Han and Kim, 2005; Bosberry and Dobson., 1989). Additionally, it was found that the overall culling rate of cows that experienced pregnancy loss was significantly higher at 46.4%, than the average at 27% (Lee and Kim, 2007). Both reproductive impacts and culling can contribute into long term costs associated with pregnancy loss.

It has been estimated that the value of pregnancy involves the difference in future income of a pregnant or non-pregnant animal. The monetary value of pregnancy alone was reported to be approximately $200 (Eicker and Fetrow, 2003). Although, this value does not include AI breeding protocol costs included in detecting estrus, resulting in a value between $253 to $274 (Stevenson, 2001). A study looking directly at value of a new pregnancy estimated pregnancy to be worth $278 (De Vries, 2006).

The simple value of pregnancy does not seem significant, yet compared to the cost of pregnancy loss, it becomes invaluable to farmers. The average cost of pregnancy loss or abortion ranges from $600 to $1,286 (Eicker and Fetrow, 2003; Weersink et al., 2002). One study found the cost of pregnancy loss of abortion to be $555 in a simulated herd, researchers estimated this value to increase with the length of gestation. Importantly, this study found that increased persistency of lactation, increased probability of pregnancy and decreased replacement heifer cost led to this decreased value, but individual milk yield was a difficult value to maintain consistency (De Vries et al., 2006). The report from this study seems exceedingly low when compared to a Korean study calculating costs including nutrition, growth for calves, production labor, medical cost, culling, and gross economic loss. When combining these factors, this study estimated the cost to be $2,333 based largely on extended calving interval costs and culling (Lee and Kim, 2007). The estimated cost of abortion can include future reproductive loss and reduced milk yield, reported in the higher estimates, rather than the immediate cost (Weersink et al., 2002).

Pregnancy Loss Risk factors -Environmental

The timing of breeding and gestation by season has been debated to be associated with pregnancy loss. It has been shown that breeding animals during a warmer season can result in exposing an early embryo to heat stress (Drost et al., 1999). At this time, embryos are more sensitive to physiologic conditions, and this may impact fetal development. There was, however, little evidence supporting pregnancy loss as a result of heat stress in the first days of pregnancy (Chebel et al., 2004).

Ealy et al., reported that embryos can become more resistant to heat stress during development at 10 days after breeding (Ealy et al., 1993). Conversely, another study reported that heat stress from 8-16 days of pregnancy can reduce fetal weight (Briggers et al., 1987). Additionally, heat stress may impact hormone levels such as prostaglandin which may also lead to early pregnancy loss (Putney et al., 1989).

Interestingly enough, a study measuring temperature-humidity index (THI) and pregnancy loss found that during 21-30 of gestation, there was a correlation between high temperatures and fetal loss. They found that additionally, these results became more significant in high producing dairy cows as there was a correlation between high producing cows and an increased risk of pregnancy loss (García -Ispierto et al., 2006). It was determined that if pregnancy was maintained by 31 days, heat stress would be unlikely to result in result in pregnancy loss (Hansen and Aréchiga, 1999). Additionally, one study examined lactating cows following Ovsynch protocols and exposed animals to heat stress. They found that there was no interaction between heat stress and insemination on pregnancy loss (Cartmill et al., 2001). At a practical level, it seems there is a window where the early embryo may be sensitive to heat stress, yet after pregnancy has been maintained for at least a month, heat stress will not play an influence on pregnancy loss.

Pregnancy loss was seen to increase with summer months as the population risk is high. Seasonal changes may include diseases, vectors, differences in feeding or endocrine function (Rensis and Scaramuzzi, 2003). In addition to heat, a study measuring a farm over a 6.5-year period, found the highest density of abortions to be in September with the lowest in October (Thurmond et al., 1990). Although the seasonality was not seen to impact late embryonic pregnancy loss (Grimard et al., 2006).

Pregnancy Loss Risk Factors- Management

Historically, farmers have aimed to increase herd milk production. However, milk production has been seen to impact pregnancy loss at varying levels. Silke et al., observed genetic merit for milk production to have a negative effect on pregnancy loss and conception rate (Silke et al., 2002). Another study by Sreenan et al., suggested that early embryonic losses are affected by genetics which includes high milk production (Sreenan et al., 2001). One study found that the incidence of pregnancy loss was over 25% of pregnant cows on days 21-24 (Michel et al., 2003). Grimard et al., did not find evidence of late embryonic loss correlated with genetics, suggesting that any genetic input regarding pregnancy loss would cause early pregnancy loss (Grimard et al., 2006). Some studies have contradicted any input from milk yield and have noted that there were no associations with late embryonic losses or conception rates (Santos et al., 2004). Overall, the results of the influence of milk yield was inconclusive.

Risk of abortion was found to be lower in heifers than in cows and second parity cows were found to have a much higher rate of abortion (Markusfield-Nir, 1997). In a study with 227 abortions, researchers found that cows at ages 3-4 years had the highest rate of abortion (Mitchell, 1960). An additional study considered the highest rate of abortion to be correlated with cows aged over 8 years (Thurmond et al., 1990).

Reoccurrence risk rate of abortion was taken in one study and was shown to increase with cow parity from a ratio of 2.5 for heifers to 3.1 for over third parity cows (Markusfield-Nir, 1997). Another study agreed that risk of culling due to reproductive failure increased with parity yet found that reproductive performance was not statistically significant (Lee and Kim, 2006). The same study found that cows with a higher parity had an increase in periparturient disorders, higher risk of retained placenta, metabolic disorder, ovarian cysts and endometritis (Lee and Kim, 2006).

In addition to disease states, physiologic levels can play an important role in pregnancy loss. When examining concentrations progesterone levels were found to have a correlation with pregnancy loss as serum concentrations on day 21 or 22 and day 23, 24, or 25 were lowest for cows that lost an embryo between days 24 and 28. Additionally, the same study found that cows with linear somatic cell count scores of > 4.5 before AI were twice as likely to lose their embryo by days 35-41 compared to those that scored less than 4.5 (Moore et al., 2005).

Genetic factors such as genetic variances can account for early pregnancy loss. One comprehensive study attributed a number of genes and gene signaling pathways as possible markers for fetal loss (Sigdel et al., 2021). Work in genetic pathways in addition to high milk yield may provide framework for selecting animals for breeding and minimizing losses.

Body condition score (BCS)

In 1919, Murray defined body condition as the ratio of body fat to nonfat components within a live animal. Further, defining body condition scoring, it is the assessment of the proportion of subcutaneous body fat an animal possesses. Body condition is evaluated commonly using a 1 to 5 scale (Wildman et al., 1982; Edmonson et al., 1989), although there are other systems used. Body condition scores provide an estimate of fat reserves but can be less accurate in very thin or very fat animals (Roche et al., 2004). The 1 to 5 scale is included to measure welfare of animals in order to accurately portray nutritional input (Defra, 2001). The lowest body condition score reflects emaciation while the highest value reflects obesity (Wright and Russel, 1984). Historically, body condition scoring has been determined by farm staff, but is limiting in reliability due to differences between training and experience. The consistency between scorers requires both time and consistent training (Edmonson et al., 1989; Ferguson et a., 1994).

Cows frequently lose condition for 40-100 days post calving due to physiological changes reflecting the increasing milk production and the negative energy balance animals experience during this period (Koenen et al., 2001; Sumner and McNamara et al., 2007).

It is well documented that during the start of lactation, cows lose body condition as there is a negative energy balance, and then increase condition as the animal can consume more energy than it requires (Broster and Broster, 1998). The dramatic decrease in body condition can be affected by factors including parity, days in milk, and previous body condition (Meikle et al., 2004). On average, the shift from negative to positive to energy balance occurs by day 63 DIM as resources can be diverted from milk production, and fat reserves are replenished (Grummer and Rastani, 2003).

It has been debated whether parity results in dramatic differences among body condition loss following birth. Some studies argue primiparous cows tend to have higher body condition scores and may see less condition lost during the first few weeks post-partum, whereas multiparous animals experience larger losses in body condition (Mao et al., 2004; Sakaguchi et al., 2009; Berry et al., 2011). Other studies, argue the recovery rate is faster in primiparous animals than multiparous animals (Ruegg and Milton, 1995). Lastly, there are papers reporting there is no differences in parity and BCS loss (Berry et al., 2007).

Body condition is known to be an indicator of production, reproduction, health, and welfare. To maintain body condition scores desired for improved management, papers cite 3.5-4.25 (5-point scale) as the optimum score for calving, although there is a discrepancy between studies, leading to the practical optimum to be between 3.0-3.5 (5 point scale) (Roche et al., 2007; Berry et al., 2007).

It is known that cows experience a lowered fertility when nutrient requirements are higher than intake, leading to a loss in body condition. Studies have shown body condition score at calving to impact future fertility, health and milk yield (Markusfield et al., 1997). The utilization of body fat reserves in order to increase milk production has been selected for through breeding (Koenen and Veerkamp, 1997; Dechow et al., 2002; Berry et al., 2003). In cases, it has been seen that a lower BCS led to a higher milk production, although in some cases it is reported they are uncorrelated (Koenen and Veerkamp, 1997).

Often the change in body condition is simply monitored at calving and AI (during lactation), but not measured at other points to determine further impacts (Garnsworthy, 2006). One study measured body condition changes across lactation and found BCS during the first stage of lactation (75d) was found to be the best predictor of fertility. The study acknowledged that they did not find a correlation between fertility and BCS across lactations, which provides conflicting information in comparison to other studies (Coffey et al., 2003; Banos et al., 2004). Studies have reported that the large standard errors have made it difficult to find a correlation between body condition and fertility, yet BCS has merit as a management and selection tool for improving fertility (Pryce et al., 2001). BCS in the first 30 days in milk can be used as an indicator of calving interval (Pryce et al., 2001). Since BCS profiles mirror lactation curves, animals with an extended calving interval have the potential to become over conditioned, which poses mortality risks (Ruegg and Milton, 1995; Heuer et al., 1999; Berry et al., 2006; Roche et al., 2007; Shahid et al., 2015). Although fertility as a whole is multifactorial, the relationship between BCS and pregnancy is correlated. Studies found that herds that got pregnant by 130 days in milk had less body condition loss, leading to fewer health problems, including an increased conception and decreased early pregnancy loss (Middleton et al., 2019).

Cows that maintain or even gain BCS after calving have a correlation with future higher fertility (Fricke et al., 2020). Thus, Fricke et al., proposed the importance of farms to monitor BCS for transition cows (3 weeks before and after calving) and at AI, additionally to utilize fertility programs including insemination periods and nutritional strategies to prevent increased BCS in late lactation, which can result in an increase of metabolic disorders (Fricke et al., 2020). Since continual monitoring of BCS and assuring condition is maintained during the transition period and in lactation, the use of automated scoring systems may bridge the gap in improving detection of BCS change.

The use of automated scoring provides the potential to aid producers in breeding and management decisions (Roche et al., 2009). Semi-automated scoring systems have been put into practice using photos, machine learning, thermal imaging, and 3D technology (Halachmi et al., 2013; Berchovich et al., 2013; Tedin et al., 2014; Tweedale and Jain, 2014; Weber et al., 2014) . However, these systems still require the assistance of well-trained management to implement them effectively. The use of a fully automated scoring system, including the DeLaval body condition scoring camera, is highly correlated with manual scoring, however there are limited studies to validate this. Automated scoring has been shown to have a lowered error rate compared to manual scoring for ideal body conditions, but regarding over or under conditioned cattle, it was less accurate than manual scoring (Krukowski, 2009; Spoliansky et al., 2016; Mullins et al., 2019). Recently a study found that the consistent monitoring of an automated body condition scoring system (ABCS) can be used to predict the change in body condition during lactation and this can be applied to formulate energy requirements to alleviate negative energy balance during the transition period (Truman et al., 2022). For the purposes of observing body condition score over time to make management and nutrition decisions, the DeLaval system shows promise (Zieltjens et al., 2020).

Body Condition and Health Outcomes

The continual monitoring of BCS and dry matter intake is critical as it can be a predictor of underlying health conditions when decreased (Hammon et al., 2006; Huzzey at al., 2007). In the case of acidosis, animals will present with a lowered body condition and a delayed recovery (Kleen et al., 2013). Additionally, it has been observed that when animals are over conditioned, they lose weight in negative energy balance at a faster rate and take longer to reach maximum milk production than those with a smaller BCS to start with (Garnsworthy and Jones, 1987; Jamali Emam Gheise et al., 2017). A study found that animals entering into parturition with extreme high or low body conditions maintained high or low body conditions post calving (Jílek et al., 2008).

Although data associating BCS and infectious diseases is minimal, it has been seen that negative energy balance and the dramatic loss of BCS can increase the risk of infectious diseases. In some studies, over conditioning was seen to have greater odds of mastitis during lactation in addition to uterine infections seen with BCS loss (Markusfeld et al., 1997; Butler and Smith, 1989; Berry et al., 2007). However, other studies suggested the relationship between infectious diseases such as metritis and BCS to have a nonlinear relationship yet agreed that extreme BCS did put animals at greater risk (Hoedemaker et al., 2009).

Subclinical disease can also be seen in association with BCS although there can be a difference across primiparous and multiparous animals. Interestingly, Berry et al., reported first and second parity animals to have a negative association with early lactation BCS, endometritis and somatic cell count, while cows in third or greater lactation had a positive relationship between BCS and somatic cell count and no relationship with endometritis (Berry et al., 2007).

Roche et al., found that younger cows may be more susceptible when thin, while older animals become immensely more susceptible to mastitis and metabolic disease when over conditioned (Roche et al., 2013). Although there is not published literature directly comparing many illnesses and BCS, it is clear that there is a relationship between BCS loss during the try period and negative health effects (Chebel et al., 2018). Since animals are in a negative energy balance immediately starting lactation, they experience a reduction of immune function, leading to approximately 75% of all diseases in dairy cows occuring in the first month of lactation (LeBlanc et al., 2006).

Changes in body condition early in lactation are associated with health events such as twinning, dystocia, retained placenta, ketosis, metritis and displaced abomasum (Ruegg and Milton, 1995; Gillund et al., 2001; Berry et al., 2007). Even in the case of one clinical disease, cows can be predisposed to other diseases concurrently and in future lactations (Dohoo and Martin, 1984). Fertility is reduced as a result of multiple clinical or subclinical diseases, resulting in reduced pregnancy at AI and pregnancy loss. It was found that clinical uterine diseases have the greatest effect on pregnancy, specifically animals that had both metritis and endometritis, in cases resulting from calcium and non-esterified fatty acid concentrations (Ribeiro et al., 2013). Since health concerns are a major risk factor of reproductive problems, health programs for diagnosis and treatment are common (LeBlanc et al., 2006). The prevalence of clinical and subclinical diseases in high producing cows is well characterized, and often are associated with reduced reproductive performance (Santos et al., 2009; 2010; Chapinal et al., 2011). In addition to diseases linked with body condition, animals are at risk for health conditions that minimize conception and pregnancy maintenance.

Health Status

Even though in recent years, there have been advancements in management protocols and breeding programs, the prevalence of disease for hypocalcemia, respiratory disease, digestive problems, retained fetal membranes and displaced abomasum still impact dairy fertility (Ribiero et al., 2013). Additionally, many illnesses such as clinical mastitis, lameness and metabolic or infectious diseases are still quite prevalent in herds and can be particularly concerning during the transition period (LeBlanc, 2010; Bacigalupo, 2017). It has been estimated that one-third of dairy cows have at least one clinical disease during the first 3 weeks of lactation, these include metritis, mastitis, digestive problems, respiratory problems, or lameness (Ribeiro and Carvalho, 2017). The estimated prevalence of illnesses was reported by USDA NAHMS, they found that producers reported mastitis (24.8%), lameness (16.8%), infertility (8.2%), and metritis (6.9%) (USDA, 2016). Unfortunately, due to the importance of health on milk yield, if animals present with clinical illnesses, there is a direct or indirect effect on milk yield reduction (Stevenson and Call, 1988).

In dairy cows, the most concerning diseases are those that pose a threat to milk production or pregnancy. Clinical and subclinical mastitis is a common disease with the potential to reoccur. Mastitis will decrease milk production and if left untreated can lead to toxic mastitis, a systemic disease (Jamali et al., 2018). Mastitis is more likely found in high producing, multiparous animals and can have a higher prevalence in farms with less consistent farm management protocols. Since mastitis frequently occurs in the beginning of a milking cycle, within the first 60 days in milk, the management and stressors the animal faces in the transition period is critical, as the animal may be more susceptible to infection (Ibrahim, 2016; Jamali et al., 2018; Hussein et al., 2018).

Health events have been shown to alter future pregnancy as health conditions are a major risk factor for depression of cyclicity. It was found that when comparing healthy and diseased animals, 84.1% of healthy cows had a normal cycle, and 70.7% of diseased animals cycled normally, leading to a decreased pregnancy rate of 34.7% compared to 51.4% of healthy cows to get pregnant at first AI. In addition, these animals were at risk for pregnancy loss within the first 60 days as healthy animals had an 8.9% of pregnancy loss and animals with a disease had a 15.8% of pregnancy loss (Santos et al., 2011; Thatcher, 2017). Santos et al., found that with a high prevalence of disease, animals were less likely to be cyclic, leading to decreased pregnancy rate and animals were more likely to lose pregnancy in the first 60d of gestation (Santos et al., 2010).

In many cases, parity plays a role in the development of health disorders and the likelihood of certain conditions and both primiparous and multiparous cows are prone to differing disorders. Overall, primiparous or lower parity animals were negatively associated with death and live culling (De Vries et al., 2010). It has been documented that primiparous cows had higher rates of still births, dystocia, and metritis than multiparous animals (Kinsel and Etherington, 1998; Bicalho et al., 2007). Multiparous animals have greater rates of twins, retained fetal membranes, subclinical ketosis, left displaced abomasum, and lameness (LeBlanc et a., 2002; Bicahlo et al., 2007; Dubuc et al, 2010; Vieira-Neto et al., 2017).

The increasing susceptibility to illness during the transition period can additionally be seen in the likelihood of uterine infections, especially infectious diseases including retained fetal membranes, metritis, and endometritis (Ghavi Hossein-Zadeh and Ardalan, 2011; Pérez-Báez et al., 2019). Within the transition period, it is possible to predict the potential of disease by measuring neutrophils, phagocytic activity, oxidative capacity, cortisol levels and interleukin expression (Kim et al., 2005; Shimizu et al., 2018). Additionally, measuring negative energy balance, feed intake, and mineral concentrations in the blood can also serve as predictors for infectious disease susceptibility (Gilbert, 2016; Braga Paiano et al., 2019). A likely source of infectious disease is retained fetal membranes when the animal does not deliver the placenta by 12-24 hours after delivering the calf. Retained fetal membranes can occur when there is a lack of coordinated function between neutrophils and macrophages, proinflammatory mediators, and detachment of the placenta (Gilbert, 2016; Patel and Parmar, 2016; Nelli et al., 2019). Patel and Parmar, 2016, researched risk factors for retained fetal membranes, or fetonomy, and found induced parturition, short gestation, abortion, twinning, dystocia, fetonomy or cesarian section were risk factors for retained fetal membranes, additionally metabolic disorders and immunosuppression can increase susceptibility for retained fetal membranes (Patel and Parmar, 2016).

The process of birth is considerably traumatic to local tissues and although inflammation is a normal part of the uterus involuting and returning to normal, it can impair fertility if the process is unregulated (Magata et al., 2016). Ideally, complete involution of the uterus should occur in a short period before first ovulation, in less than 21d, for animals to have a normal estrus cycle and pregnancy (Thatcher et al., 2006). Metritis and endometritis specifically involve infection affecting the layers of the uterus after 21 days in milk. Metritis, which effects all layers of the uterus, can be seen clinically as watery discharge with a red or brown appearance and additionally the animal can present with systemic clinical signs (Sheldon et al., 2006; 2008). Endometritis, effecting only the lining layer of the uterus, will have discharge containing purulent or mucopurulent material after 3-4 weeks post-partum (Sheldon et al., 2006). An additional condition possible is pyometra, involving a uterus with purulent material after a corpus luteum persists and is unable to exit due to a closed cervix (Sheldon et al., 2006; 2008). Pinedo et al., reported that the magnitude of disease effects on fertility has not been fully documented, including the environmental stressors leading to fertility problems (Pinedo et al., 2020).

The importance of uterine infections such as endometritis, metritis and pyometra is the increased risk of pregnancy loss and the future association with future decreased fertility. Clinical infections can lead to reduced fertilization, development of follicles, reduced embryo implantation and fetal growth (Ribeiro, 2016; 2017; Velázquez et al., 2019). It was reported that the detrimental effects of disease in early lactation can lead to subsequent problems with conception and embryo survival up to 4 months after the initial disease (Ribeiro et al., 2016; Carvalho et al., 2019). Ultimately, as a result of disease during lactation, cow performance is reduced leading to an increased risk of death and culling (De Vries et al., 2010). It has been proven that ovarian cyclicity and pregnancy at first AI are affected by both reproductive and non-reproductive diseases during lactation (Pinedo et al., 2020).

Final remarks

Maintaining pregnancy until term is an important aspect to dairy farm profitability and sustainability. In the last several years, efficacy in dairy production has advanced to the point where the number of animals has decreased, and milk production has increased. Unfortunately, there are challenges, specifically during the transition period that leads animals to become susceptible to disease, leading to reduced reproductive capabilities. Pregnancy loss poses a hazard to future health and productivity for both primiparous and multiparous cows, so understanding risk factors leading to pregnancy loss is a critical factor to improve reproductive performance within the dairy industry. The objective of this thesis was to identify and understand risk factors of early pregnancy loss such as body condition score, change in body condition.

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CHAPTER 2: ASSOCIATION BETWEEN BODY CONDITION SCORE FLUCTUATIONS AND PREGNANCY LOSS IN HOLSTEIN COWS

INTERPRETIVE SUMMARY

Maintenance of the pregnancy until term is a key factor for adequate fertility in dairy operations, as early embryonic mortality in lactating dairy cows is significant. The objective of this study was to investigate the association between the changes in body condition scores (BCS) during early lactation and in the proximity of artificial insemination (AI) and embryo mortality in Holstein cows. A secondary objective was to determine the impact of disease on pregnancy loss, considering multiple time periods relative to artificial insemination. Our study population included 9,430 lactations in 6,884 Holstein cows that had their daily body condition scores determined by an automated camera system during early lactation and close to AI. Cows were diagnosed pregnant via transrectal ultrasonography on d 32 after AI and reconfirmed at d 80 after AI. Overall, the dynamics of BCS differed between cows that lost or did not lose their pregnancy. Low BCS and more pronounced reductions in BCS occurring closer to the artificial insemination, as well as concurrent disease, resulted in greater levels of pregnancy loss.

OVERVIEW

The objective of this study was to characterize the associations between body condition score (BCS) and BCS change (ΔBCS), determined by an automated camera system during early lactation and close to AI, and the subsequent pregnancy loss (PL) in Holstein cows. A secondary objective was to determine the impact of disease on PL, considering multiple time periods relative to AI. Data from 9,430 lactations in 6,884 Holstein cows in a commercial dairy operation

located in Colorado, USA were included in this retrospective observational study. Cows were subject to first AI at about 80 DIM (primiparous) and 60 DIM (multiparous), following a double OvSynch protocol. Pregnancy diagnosis was performed via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 after AI. Cameras mounted on the sorting-gate at each exit (n = 2) of the milking parlor generated BCS in a 5-point scale with 0.1 increments. The BCS at calving (BCS1), 21 DIM (BCS21), 56 DIM (BCS56), AI resulting in pregnancy (BCSAI), and 90 d post AI (BCSAI90) were selected for the analyses and subsequently categorized as low (≤ lower quartile), moderate (interquartile range), and high (≥ upper quartile). Changes in BCS were calculated by periods of interest as change from calving to 21 DIM; change from calving to 56 DIM; change from 56 DIM to AI; and change from AI to 90 d post AI and assigned into categories to facilitate the analysis. Data were examined using logistic regression, considering parity category, season at calving and at AI, DIM at AI, milk yield up to 60 DIM, and occurrence of disease as covariables. The logistic regression analyses indicated that the odds of PL were greater in cows in the low BCS category relative to cows in the high BCS category at 56 DIM (OR 95% CI = 1.41 [1.12-1.79]), AI (1.31 [1.05-1.65]), and 90 d post AI (1.38 [1.10-1.74]). Likewise, cows with large loss in BCS between calving and 21 DIM (1.46 [1.10-1.94]) and loss in BCS between AI and 90 d post AI (1.44 [1.15-1.81]) had greater odds of PL compared to cows with no loss of BCS within the same period. Occurrence of disease at all the time periods considered in in the analysis had a consistent detrimental impact on maintenance of pregnancy, supporting the concept that pre and postconceptional disease affects embryonic survival. Overall, low BCS, more pronounced reductions in BCS occurring closer to AI, and occurrence of disease resulted in greater pregnancy loss in this Holstein population.

INTRODUCTION

The significant role of adequate fertility in the sustainability of dairy operations through impacts on milk production, genetic gain, and culling policies has been widely recognized (Britt, 1985; De Vries, 2006). Reproductive efficiency in dairy herds mainly depends on proper submission of lactating cows to AI in a timely manner with adequate probability of pregnancy per AI (P/AI) (Ribeiro et al., 2016). However, maintenance of the pregnancy until term is also a factor to consider, as early embryonic mortality in lactating dairy cows is significant (Santos et al., 2004; Diskin et al., 2006).

The success of pregnancy establishment and maintenance until calving is influenced by multiple factors. Causes of pregnancy loss (**PL**) in dairy cows include genetic components, infectious pathogens, and environmental factors, which result in suboptimal uterine conditions and less competent embryos (Moore et al., 2005; Wiltbank et al., 2016). Moreover, anovulation before synchronization of the estrous cycle (Santos et al., 2004) and inflammatory diseases occurring before breeding (Santos et al. 2010; Ribeiro et al., 2013, Pinedo et al., 2020) have also been implicated with increased early embryonic mortality. Remarkably, the impact of factors such as heat stress on PL is generally underestimated, as embryo losses occurring during the first days post conception are undetected and only manifested through reduced conception at the first pregnancy diagnosis (Wiltbank et al., 2016).

The impact of inadequate body condition during early lactation in fertility has also been explored (Lopez-Gatius et al. 2002; Roche et al., 2009; Barletta et al., 2017). As the increased demand for nutrients to support the initiating lactation is followed by slower increments in DMI (Gross et al., 2011), a significant proportion of lactating dairy cows lose body mass postpartum. lactating dairy cows usually lose significant amounts of body mass postpartum. This mobilization of fat and labile protein from body energy reserves, including the deposits of subcutaneous fat, has consequences on the health and performance of the early lactation cow; however the underlying mechanisms behind this association are not fully understood (Bauman and Currie, 1980; Lean et al., 2013). Interestingly, the magnitude in the change in BCS following dry-off has also been established as a relevant factor impacting subsequent fertility and health. As reported in recent studies (Carvalho et al., 2014; Chebel et al., 2018; Melendez et al., 2020), cows that lost BCS during the late dry period had increased occurrence of postpartum disease with greater culling and death during the first three month of lactation.

Santos et al. (2009) reported that cows with extensive reductions in body condition score (**BCS**) between parturition and AI following synchronized ovulation had extended anovulatory periods, decreased P/AI, and increased risk of PL, compared with cows that had less than one unit loss of BCS or no loss in BCS. Ribeiro et al. (2016) reported similar results for cows with low BCS (BCS < 3.0) at the moment of AI, compared to those with moderate BCS (BCS ≥ 3). Nonetheless, the impacts of both low BCS or loss of BCS on PL have not been widely explored and some authors have failed to fully demonstrate these associations (Barletta et al., 2017; Manríquez et al., 2021).

Connected with the inherent nutritional imbalances occurring during the transition period and despite management efforts, significant proportions of high producing dairy cows develop metabolic or infectious diseases in the first month of lactation (LeBlanc et al., 2006, 2010). Nonetheless, the interrelationship between loss in BCS and occurrence of disease is complex and establishing precise cause and effect associations is challenging. As for inadequate BCS, the negative impact of disease events on maintenance of pregnancy has been described (Santos et al., 2010; Ribeiro et al., 2013). Notably, the detrimental effects of health conditions occurring during early lactation extend beyond this period, affecting the development of the early conceptus as well as the maternal recognition process (Ribeiro et al., 2016; Carvalho et al., 2019).

Our hypothesis was that the effect of insufficient body reserves, as well as pronounced losses in BCS would be relevant factors increasing PL when occurring postpartum and close to AI. Therefore, the objective of this study was to characterize the associations between BCS and BCS change (**ΔBCS**), determined by an automated camera system during early lactation and close to AI, and the subsequent pregnancy loss in Holstein cows. A secondary objective was to determine the impact of disease on PL, considering multiple time periods relative to AI.

MATERIALS AND METHODS

Study Design and Study Population

The dataset analyzed in this retrospective observational study originated from a previous work investigating the association between BCS and pregnancy at the first AI postpartum (Pinedo et al., 2022). Data were collected from 9,430 lactations in 6,884 Holstein cows calving between April 2019 and March 2021 in a commercial dairy operation located in Windsor, Colorado, USA. For the current research, only cows that were diagnosed pregnant at 32±3 after AI were included in the analyses.

Details on cow management were provided in Pinedo et al. (2022). Briefly, cows were maintained in a cross ventilated barn, milked 3X in a 90 units rotary parlor and subject to first AI at about 80 days in milk (**DIM**) (primiparous) and 60 DIM (multiparous), following a double OvSynch protocol. Briefly, cows received GnRH at 53/33 ± 3 DIM (primiparous/multiparous), followed by an injection of PGF 7 d later and GnRH 72 h after PGF, then began the Ovsynch-TAI protocol 7 d later. The Ovsynch protocol consisted of GnRH at 70/50 ± 3 DIM (primiparous/multiparous), PGF 7 d and 8 d later, GnRH 56 h after first PGF, and AI 16 to 20 h later (modified from Souza et al., 2008).

Pregnancy diagnosis was performed by trained farm personnel via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 of gestation. Pregnancy was identified by the detection of an embryonic vesicle with a viable embryo based on presence of heartbeat. Cows determined non-pregnant were administered prostaglandin F2α when a corpus luteum was visible and were submitted for AI based on estrus detection using the DeLaval activity meter system (DelPro Farm Manager software). Non-pregnant cows with no visible corpus luteum were submitted for AI based on estrus detection without any intervention.

Data collection started at calving and continued until reconfirmation of pregnancy at d 80±3 of gestation. Cow demographic, reproductive, and health data were extracted from on-farm software (Dairy Comp 305; Valley Ag Software, Tulare, CA). Daily milk yield and BCS were extracted from DelPro Farm Manager software (DeLaval International AB, Tumba, Sweden). The dataset included cow ID, date of calving, lactation number, calving-related and disease events, insemination dates, pregnancy diagnosis outcomes, daily milk yield for the first 60 DIM, and daily BCS.

Body Condition Scoring and BCS Categorization

Three scores per day, following the 3 daily milkings were generated by an automated BCS system (DeLaval International AB, Tumba, Sweden) and combined in one daily score. The system, previously validated by Mullins et al. (2019), included video cameras that were mounted on the sorting-gate at each exit (n = 2) of the milking parlor (Pinedo et al., 2022). A continuous video was taken as each cow walked through the parlor exit and a 3D image was created and processed through an algorithm (Mullins et al., 2019) that considered physical characteristics of the cow to calculate the automated BCS. The proprietary algorithm used the BCS scale proposed by earlier studies (Ferguson et al., 1994) ranging from 1 (emaciated) to 5 (fat), modified to report BCS in 0.1 instead of 0.5 increments.

All automated BCS data were recorded in and downloaded from DelPro Farm Manager. Specific time points were chosen based on previously reported associations between BCS and reproductive outcomes (Santos et al., 2009; Roche et al., 2009; Carvalho et al., 2014; Pinedo et al., 2022). The BCS concurrent with critical events (calving and AI), as well as BCS at time points reflecting the cows' condition during the transition period were also considered. Selected points included BCS at calving (**BCS1**), 21 DIM (**BCS21**), 56 DIM (**BCS56**), AI (**BCSAI**), and 90 d post AI (**BCSAI90**).

As BCS originated by the camera system were not reported as a continuous variable, but at 0.1 intervals, scores were categorized using the quartile distribution at each time point in analysis. Individual cow values for BCS at each time (BCS1, BCS21, BCS56, BCSAI, and BCSAI90) were categorized as low (≤ lower quartile), moderate (interquartile range), and high (≥ upper quartile), separately for primiparous and multiparous cows (Pinedo et al., 2022). Changes in BCS were calculated for each cow by periods of interest subtracting the BCS at the earliest time from the BCS at the latest time as follows: Δ calving to 21 DIM = BCS21 - BCS1; Δ calving to 56 DIM = BCS56 - BCS1; Δ 56 DIM to AI = BCSAI – BCS56; and Δ AI to 90d post AI = BCSAI90 - BCSAI. Furthermore, based on the numerical difference between time points, cows were assigned into one of the following categories for Δ calving to 21 DIM and Δ calving to 56 DIM: large loss of BCS (top 25% of cows loosing BCS); moderate loss (bottom 75% of cows loosing BCS); or no loss (ΔBCS ≥ 0). Because the proportion of cows with BCS loss in the periods Δ 56 DIM to AI and Δ AI to 90d post AI were smaller, the large and moderate BCS loss categories were merged into one BCS loss category for these two periods.

Other Explanatory Variables and Study Outcome

Calving-related and disease events were obtained from farm records stored in on-farm software (Dairy Comp 305). Reproductive health events of interest included retained fetal membranes (RFM; membranes not expelled after 24 h postcalving, Kelton et al., 1998), metritis (7 ± 3 DIM; watery, reddish/brownish fetid discharge, independent of fever; McDougall et al., 2007), and pyometra (25 ± 3 DIM/at pregnancy checking; ultrasound examination evidencing accumulation of purulent material within the uterine lumen in the presence of a persistent corpus luteum and a closed cervix, Sheldon et al., 2006). Non reproductive disorders included clinical hypocalcemia (down cow or cow unsteady prior to calving to 1 or 2 days after calving with no other abnormal physical exam findings and responsive to calcium administration), subclinical ketosis (5 ± 3 DIM; blood BHB >1.3 mmol/L), left displaced abomasum (off feed, scant pasty manure, ping in left flank, usually within 30 DIM), lameness (assessed weekly; score >2; Bicalho et al., 2007), clinical mastitis (abnormal milk or udder inflammation); digestive problem (off feed, altered feces), injury (visible body trauma including wounds, ulcerations, and swelling), and pneumonia (nasal discharge, respiratory distress, altered lung sounds).

Three different health categorizations were created for the analysis. First, the variable disease considered disease events diagnosed between calving and 80 DIM, categorized into reproductive (**REP80**; retained fetal membranes, metritis, and pyometra) or other disorders (**OTH80**; clinical hypocalcemia, clinical ketosis, left displaced abomasum, lameness, clinical mastitis, digestive problem, injury, and respiratory disease). Based on this information, lactations were classified as REP80, OTH80, both, or no events recorded (healthy). Second, the variable disease within 90 d after AI was created considering diseases occurring between the AI resulting in conception and 90 d post AI, grouping cows as healthy or sick (pyometra, lameness, clinical mastitis, digestive problem, injury, and respiratory disease).

Parity category was created as a binary variable including primiparous (lactation number =1) and multiparous (lactation number ≥2) cows. Calvings and AI were grouped by season (spring, summer, fall, or winter). Days in milk at AI were categorized as low (≤ 90 DIM); medium (91 to 150 DIM); and high (>150 DIM). Finally, a milk yield category was added as a covariable in the models using the quartile distribution of the average daily milk yield in the first 60 DIM (**M60**) obtained from DelPro Farm Manager. Cows below the lower quartile were classified as low M60 (≤31.1 kg), cows between the lower and upper quartile were classified as medium M60 (31.1-46.6 kg), and cows in the upper quartile of M60 were classified as high M60 (>46.6 kg).

The study outcome was PL, assessed via transrectal ultrasonography at d 80±3 of gestation. Cows diagnosed pregnant at first examination and subsequently diagnosed not pregnant at the following examination were considered to have undergone pregnancy loss.

Statistical Analyses

An initial screening of the variables potentially associated with PL was completed using univariable models. Subsequently, multivariable models that included BCS, ΔBCS, or disease occurrence were tested considering parity category, season at calving and at AI, DIM at AI, and milk yield up to 60 DIM as covariables. Least square means for BCS and ΔBCS at specific time points by parity category and occurrence of PL (Table 2.2 and Table 2.3) were calculated using ANOVA (PROC GLM; SAS institute Inc., Cary, NC).

Odds ratios (**OR**) and predicted probabilities for PL were calculated for the explanatory variables using PROC LOGISTIC (Table 2.4 and Table 2.5). A backward stepwise selection approach was used considering the categories of BCS (high reference) and the categories of ΔBCS (no loss reference), the covariables (**COV**), and their first order interactions in the initial model. The logistic equation to investigate the association of ΔBCS and the outcome variables of interest can be expressed as presented by de Mutsert et al. (2009):

i) BCS at time points of interest:

$$\ln [p/(1- p)] = \beta_0 + \beta_1(\text{BCS}) + \beta_2(\text{COV}) + \beta_3(\text{BCS x COV})$$

ii) ΔBCS of periods of interest:

$$\ln [p/(1- p)] = \beta_0 + \beta_1(\Delta\text{BCS}) + \beta_2(\text{COV}) + \beta_3(\Delta\text{BCS x COV})$$

where ln is the natural logarithm, p is the proportion of cows with PL and $[p/(1 - p)]$ is the odds of this outcome, β_0 is the model intercept for the outcome of interest, β_1, β_2, and β_3 are the regression parameters for BCS, ΔBCS, the covariables, and the interaction terms BCS x COV or ΔBCS x COV.

From the final logistic model, predicted probabilities and 95% confidence intervals (**CI**) were calculated using the p=pred and the l=lower and u=upper options in the output statement of the procedure. Predicted probabilities for PL were modeled using PROC GENMOD with BCS and ΔBCS categories as predictors (Figure 2.1).

The distribution of PL by disease category and the effects of parity category, season and DIM at AI, and disease were evaluated by logistic regression (Table 2.6). Daily BCS least square means were calculated by PL outcome (pregnant at 80±3 of gestation reexamination vs. open at reexamination), and parity and presented as BCS curves from calving to 200 DIM (Figure 2.2). For all outcome variables, significant predictors were selected at P -value <0.05; interaction terms and controlling variables remained in the models at P -value ≤0.10.

RESULTS

The dataset included 9,430 lactations (primiparous = 4,034; multiparous = 5,396) in 6,884 Holstein cows that were diagnosed pregnant at 32±3 after AI. A total of 3,240, 3,675, 3,782, 3,638, and 3,203 lactation records had BCS at calving, 21 DIM, 56 DIM, AI, and 90 d post AI in primiparous cows. For multiparous cows, 4,205, 4,656, 4,758, 4,815, and 4,135 lactation records with BCS at calving, 21 DIM, 56 DIM, AI, and 90 d post AI were available.

Overall, the distribution of inseminations across seasons were spring 15.9%, summer 36.2%, fall 27.8%, and winter 20.1%. Mean (SD) DIM to AI of conception for primiparous and multiparous cows were 109.9 d (Q1 = 80 d, median = 83 d, and Q3 = 121 d) and 104.1 d (Q1 = 60 d, median = 87 d, and Q3 = 128 d; P =< 0.001), respectively. The overall incidence of pregnancy loss was 9.95%, with primiparous cows evidencing lower PL than multiparous cows (6.87% vs. 12.2%; P <0.0001).

Mean BCS values consistently decreased across time points from calving to 56 DIM (Table 2.1 and Table 2.2) and the greatest reductions in BCS occurred in multiparous cows between calving and 56 DIM (Table 2.3). From this point, mean BCS started to increase, with maximum values at 90 d post AI.

The differences in BCS least square means (LSM) between cows that maintained or lost their pregnancy were variable, depending on the time of scoring and the model used (univariable vs. multivariable, Table 2.2). The univariable comparisons for primiparous cows indicated that

BCS at 21 DIM (3.18 vs. 3.20) and 56 DIM (3.11 vs. 3.14) were lower in cows with PL. However, the full model dismissed the differences at 21 DIM. In multiparous cows, BCS were lower at 90 d post AI in cows with PL for both the univariable (3.17 vs. 3.20) and the multivariable (3.19 vs. 3.23) models (Table 2.2).

When ΔBCS were compared between cows with and without PL, according to the univariable analysis, primiparous cows with PL had a slightly greater increase in BCS from 56 DIM to AI (0.06 vs. 0.04). In multiparous cows, greater loss and smaller gain of body condition was determined in PL from calving to 21 DIM (multivariable model -0.22 vs. -0.20) and from AI to 90 d post AI (0.11/0.13 vs. 0.14/0.16 univariable/multivariable model), respectively (Table 2.2).

Differences in the curves for the average daily BCS during the first 200 DIM were more evident in primiparous cows when lactations were grouped by PL (Figure 2.2). Cows that lost their pregnancy had lower BCS starting at the second week postpartum, until the end of the monitoring at 200 DIM.

The predicted probabilities of pregnancy loss by category of BCS and ΔBCS are presented in Figure 1, which provides a more intuitive interpretation of the associations between these variables. Only cows evidencing low BCS at 56 DIM (0.11 vs. 0.08), d of AI (0.10 vs. 0.08), and 90 d post AI (0.12 vs. 0.08) had greater probabilities of PL compared with cows in the high BCS category. For ΔBCS, differences in PL were established between the large loss and the no loss categories for Δ calving to 21 DIM (0.10 vs. 0.08), between large loss and the moderate loss categories for and Δ calving to 56 DIM (0.11 vs. 0.09), and between loss and no loss in the period Δ AI to 90d post AI (0.13 vs. 0.09).

The effects of the interactions of parity by BCS and parity by ΔBCS on PL were not significant and therefore the results from the logistic regression analyses are presented for all the lactations combined, including parity category in the model. The associations between BCS category and PL were more evident closer to AI, with greater odds of PL in cows with low BCS at 56 DIM, AI, and 90 d post AI (Table 2.4). When ΔBCS was considered, cows with large BCS loss between calving and 21 DIM and cow with BCS loss between AI and 90 d post AI had greater odds of PL compared with cows with no BCS loss (Table 2.5).

The distribution of cows by PL and disease occurrence at multiple periods is presented in Table 2.6. The proportions of cows with PL were different among disease categories for the 3 time periods analyzed in the study, in both primiparous and multiparous cows.

The associations among other potential factors affecting PL are presented in Table 2.7. The odds of PL were greater in multiparous cows compared with primiparous cows (OR [95%CI] = 1.71 (1.47-1.99). The odds of PL were greater for winter AI compared with summer AI (1.39 [1.12-1.74]) and pregnancies resulting from AI after 150 DIM had greater odds of PL compared with AI ≤90 DIM (1.30 [1.08-1.57]). Occurrence of disease from calving to 80 DIM, from calving to 90 d post AI, and between AI and 90 d post AI were consistently associated with increased odds of PL.

DISCUSSION

A significant proportion of lactating dairy cows lose body condition after parturition (Bauman and Currie, 1980; Britt, 1992; Truman et al., 2022). Nonetheless, as reported by Middleton et al. (2019), some cows would maintain or gain BC during the first month of lactation. In the current study, mean BCS values consistently decreased across time points from calving to 56 DIM. Nonetheless, the small magnitude of the ΔBCS from calving to 21 DIM at Q3 (-0.10) indicates that a proportion of the cows had no loss of BC during this period.

The detrimental effects of low BCS and severe loss of BCS during early lactation in reproductive indicators, such as early resumption of ovarian cyclicity and pregnancy rate have been described (Roche et al., 2009; Carvalho et al., 2014; Pinedo et al., 2020; 2022). Reductions in BCS during the dry period have also been associated with lower proportions of cyclic cows at 50 DIM (Barletta et al., 2017) and decreased pregnancy after first and second AI (Chebel et al., 2018). Moreover, cows that lost BCS during the late dry period had increased odds of being diagnosed with several postpartum diseases and evidenced greater culling and death during the first 90 DIM (Melendez et al., 2020).

To a lesser extent, the effect of BCS in maintenance of pregnancy has also been explored. Lopez-Gatius et al. (2002) reported that the risk of PL increased 2.4-fold for each unit decline in BCS from calving to 30 days postpartum. Similarly, Santos et al. (2009) reported that cows with greater BCS at calving and AI, as well as cows with no change or that lost <1 unit of BCS from calving to AI had a reduced risk of PL. In agreement, Carvalho et al. (2014) established that cows with significant BCS loss during early postpartum displayed increased impairments of embryo development during the first week after AI. Interestingly, in a more recent study, Middleton et al. (2019) reported that cows that lost BC during the first 30 DIM were at greater risk of losing their pregnancy from 35 and 60 d after first AI (0.0 vs. 6.7%) compared with cows that maintained or gained body condition.

The aim of the current study was to expand the scope of these previous findings, considering the advantage of daily BCS originated from an automated camera system. This system provides high frequency data from a large population of cows, objective measures without human error, and flexibility in the selection of specific time points of interest, such as the end of the transition period, the day of AI, and the days following conception.

Previous research has identified the peripartum and the early lactation as critical periods for subsequent fertility. Although the mechanisms resulting in increased PL in cows with greatest BCS loss following parturition are not yet defined, it has been suggested that follicles exposed to severe negative energy balance would have impaired development, producing inferior quality oocytes and dysfunctional corpora lutea (Britt, 1992). Furthermore, recent studies suggests a significant role for the active uptake of fatty acids into the oocyte during the early postpartum period, which would affect the subsequent early embryonic development (Leroy et al., 2005; Aardema et al., 2011). In partial disagreement, findings from this study do not suggest that BCS during early post-partum had a clear impact on subsequent pregnancy loss. It was only closer to AI (BCS56, BCSAI and BCSAI90) that cows in the low BCS category had greater odds of PL relative to the high BCS category (Table 2.4). Nonetheless, when ΔBCS were analyzed, large losses in BCS from calving to 21 DIM were also associated with increased pregnancy loss (Table 2.5).

Additionally, stressors or disease events occurring in the proximity of conception could be detrimental to the early embryogenesis (Hansen et al., 2004; Hernandez et al., 2012) and disruptions of the uterine environment, with increased endometrial expression of markers of inflammation, have been proposed as potential causes for pregnancy loss (Wathes et al., 2007). In consequence, our attention was not only focused on calving and early lactation, but also in periods around the time of conception (day of AI and 90 d post AI), where inadequate BCS could also impact embryo survival.

The embryo losses during early gestation represent about 12% of the total losses in high producing cows, in which abnormal placentation and embryonic development defects commonly occurs (Wiltbank et al., 2016). In the current study, PL was 9.95% (primiparous = 6.87%; multiparous = 12.2%), which is within the range of values previously reported in Holstein cows (Moore et al., 2005; Hernandez et al., 2012; Pinedo et al., 2020). For example, PL values in our study are like those presented in a multistate analysis where PL at first AI in 4,098 primiparous and 7,631 multiparous cows were 8.44% and 11.1% (Pinedo et al., 2020). Similarly, Santos et al. (2009) reported 8.7% and 17.2% of PL in primiparous and multiparous cows, respectively, with PL ranging from 11.3% to 20.5% among the 4 participant farms.

The BCS originated by the camera system were not reported as a continuous variable, but at 0.1 intervals. Although this represents a limitation for the statistical analysis, in practical terms a difference smaller than 0.1 points in body condition would be difficult to assess. In our study, mean BCS decreased from calving to 56 DIM with the greatest reductions in BCS evidenced in multiparous cows between calving and 56 DIM. From this point, mean BCS started to increase, with maximum values at 90 d post AI. Consistent with these findings, a recent study exploring the dynamics of BC through the lactation, reported that the average time for primiparous and multiparous cows to the nadir BCS was 38 and 54 d, with an average BCS loss of 0.14 and 0.3 points, respectively. Subsequently, the study cows recovered the lost BCS after 256 d (Truman et al., 2022).

In the study reported here, the differences in BCS between cows that maintained or lost their pregnancy were variable across time points. Considering the full model, mean BCS at 56 DIM was lower in primiparous cows with PL than in cows that maintained their pregnancy, while in multiparous cows this difference was identified at 90 d post AI (Table 2.2). Only in multiparous cows, a smaller gain of BC was determined in cows with PL from AI to 90 d post AI (Table 2.3). When the average daily BCS for the first 200 DIM were presented by PL status, the differences in the curves were more evident in primiparous than in multiparous cows (Figure 2.2). In this subpopulation, cows that lost their pregnancy evidenced lower BCS starting at the second week postpartum, until the end of the monitoring at 200 DIM.

The associations between BCS category and PL were more evident closer to AI, with greater odds of PL in cows with low BCS at 56 DIM, AI, and 90 d post AI (Table 2.4 and Figure 2.1). When ΔBCS was considered, cows with large BCS loss between calving and 21 DIM and cows with BCS loss between AI and 90 d post AI had greater odds of PL compared with cows with no BCS loss (Table 2.5 and Figure 2.1).

The impeding effect of inadequate BCS during early lactation in resumption of ovarian cyclicity is widely recognized (Lopez-Gatius et al. 2003; Roche et al., 2009; Santos et al., 2009). Relevant to our findings, the effect of anestrus in pregnancy loss has also been identified (Galvão et al., 2004; Santos et al., 2004; 2009). The proposed mechanisms for the effect of anovulation in PL include reduced concentrations of estradiol (Butler, 2003) and both lack of adequate progesterone in the preceding estrous cycle to AI and lesser concentrations of estradiol during proestrus resulting in premature luteal regression (Mann and Lamming, 2000). Although information on the time at resumption of post-partum cyclicity was not available for the current analyses, it is plausible to infer that cows losing BCS early in lactation would be at greater risk of anestrus, which could result in increased PL in this subpopulation of cows. Nonetheless, as cows in this farm were submitted for first AI using a double Ovsynch protocol, the increased presence of a corpus luteum at the beginning of the second Ovsynch would likely weaken this association (Souza et al., 2008).

In a recent study from our group (Manriquez et al., 2021), the magnitude of the reduction in BCS during the first 40 DIM cows was significantly associated with the probability of PL at 60 d of gestation. In agreement with our findings, the probabilities of PL in cows with excessive (ΔBCS ≤ -0.75), moderate (ΔBCS = -0.5 to -0.25), no change or gain in BCS were 0.08, 0.069, 0.067, and 0.056 respectively. Supporting these findings, Carvalho et al. (2014) reported that in cows in a multiple ovulation treatment the percentage of fertilized oocytes that were transferable embryos was less for cows that had the largest amount of postpartum body weight loss. Moreover, the percentage of degenerate embryos was the greatest in this group of cows, indicating a reduction in embryo quality and an increase in degenerate embryos by day 7 after AI.

The association between low BCS close to AI and PL identified in this study, as well as in previous research (Santos et al., 2009), could be associated with low levels of micronutrients including glucose, arginine, trace minerals and fatty acids, and growth factors (IGF-1) because of suboptimal nutritional status (Butler, 2003; Ribeiro et al., 2015). Moreover, as depicted in Figure 2, mean BCS were improving by the time of conception, and cows in the low BCS category may have been affected by concomitant issues. Interestingly, these effects could be underestimated, as PL would be misclassified as failure to conceive if the embryo is lost before pregnancy has been diagnosed (before 32 d post AI in the case of this study). In this regard, the current results align with the reported effect of inadequate BCS in P/AI reported elsewhere (Roche et al., 2009; Carvalho et al., 2014; Pinedo et al., 2022).

To our knowledge, the impact of BCS and ΔBCS post conception on PL have not been widely explored. Interestingly, at 90 d post AI, cows in the low BCS category had greater predicted probabilities of PL compared with cows in the medium and high BCS categories.

Similarly, cows that lost body condition during the 90 days following AI had greater probabilities of PL than cows with no body condition loss. As submission to AI in this population occurred after the point where average cows start recovering BCS (Table 2.2 and Figure 2.2), it is possible that the individuals identified losing body condition after AI may have been affected by concurrent issues, including disease. Nonetheless, temporal analysis between the changes in BCS and occurrence of disease would be necessary to establish causation relationships.

New research on the dynamics of adipose tissue stored in areas other than the subcutaneous adds complexity to the associations described here. The similarities between the fatty acid profiles of abdominal fat and NEFA suggest that abdominal fat is preferentially mobilized in dairy cows under negative energy balance. Consequently, cows favoring the deposit and mobilization of fat in the abdominal cavity would be at greater risk of releasing excesses of NEFA, developing more diseases, such as fatty liver, LDA, and/or ketosis (Hostens et al. 2013).

Compromised postpartum health has a negative effect on performance and survival of dairy cows and occurrences of uterine, metabolic, and other health disorders have been identified extensively as risk factors for lower subsequent fertility and milk yield and higher risk of culling (Santos et al., 2010; Ribeiro et al., 2013; Carvalho et al., 2019; Mohtashamipour et al., 2020). In this study, occurrence of disease from calving to 80 DIM, from calving to 90 d post AI, and between AI and 90 d post AI were consistently associated with increased odds of PL (Table 2.6 and Table 2.7). Increased concentrations of acute phase proteins and inflammatory mediators, such as haptoglobin and cytokines during the postpartum have been associated with impaired luteal development resulting in greater PL (Colazo et al., 2016; Strüve et al. 2013; Sina et al., 2018). Moreover, the potential downstream consequences of these changes would include cell activation, particularly immune cells, and potential problems with tissue rejection by the immune system, which could explain a potential rejection of the conceptus and PL (Ribeiro et al., 2016). The effects of inflammatory diseases before AI on the future embryo are recognized and include reduced cleavage and survival of zygotes to the morula stage, impaired conceptus elongation, reduced secretion of IFN-τ during the period of pregnancy recognition, distinct responses of ISG in PBL at onset of implantation, and increased PL (Ribeiro et al., 2016).

In agreement with these findings, in a recent study PL at first AI was increased by reproductive disorders (Pinedo et al., 2020), while Carvalho et al. (2019) and Ribeiro et al. (2016) presented evidence on the carryover effects of inflammatory disease occurring before AI that resulted in impaired development of the early conceptus and maternal recognition in the uterine lumen. Nonetheless, disease events occurring in the proximity of conception could also result in PL through the release of lipopolysaccharide, proteoglycans, and molecules of bacterial origin that activate inflammatory and immune responses (Hansen et al., 2004; Hernandez et al., 2012). The resulting increase in cytokines could affect embryo survival by disrupting function of the hypothalamus, pituitary, ovaries, and uterus. Supporting this idea, multiple reports have identified the negative effect of clinical mastitis close to AI on maintenance of pregnancy (Risco et al., 1999; Chebel et al., 2004; Hernandez et al., 2012, Dahl et al., 2020).

The findings reported in this study highlight the potential for adding information from automated technologies into the on-farm decision making process. Although the detrimental effect of excessive BCS loss post calving has been widely recognized, identifying specific time points that are more relevant as predictors of subsequent fertility may result in more efficient monitoring programs. This information indicative of the cow energy status and energy balance could be considered for individual-level decisions, such as time of AI and type of semen to be used or aggregated for consideration in management policies at the herd level.

CONCLUSIONS

The effects of low BCS and loss in BCS on PL were moderate and more evident when occurring closer to AI. Occurrence of disease in all the time periods in the analysis had a consistent detrimental impact in maintenance of pregnancy, supporting the concept that pre and postconceptional disease affects embryonic survival.

TABLES AND FIGURES

Table 2.1. Descriptive statistics for BCS at multiple time points and time periods by parity category.

Time point (BCS)		Q1	Median	Q3	Mean	SD
Primiparous	Calving	3.30	3.40	3.50	3.35	0.16
	21 DIM	3.10	3.20	3.30	3.19	0.17
	56 DIM	3.00	3.20	3.30	3.13	0.18
	Day of AI1	3.10	3.20	3.30	3.18	0.18
	90 d post AI	3.20	3.30	3.40	3.27	0.18
Multiparous	Calving	3.20	3.30	3.50	3.31	0.21
	21 DIM	3.00	3.10	3.30	3.11	0.21
	56 DIM	2.90	3.00	3.10	2.99	0.23
	Day of AI1	2.90	3.10	3.20	3.05	0.23
	90 d post AI	3.00	3.20	3.35	3.19	0.23
Overall	Calving	3.20	3.40	3.50	3.33	0.19
	21 DIM	3.00	3.20	3.3.0	3.16	0.20
	56 DIM	2.90	3.10	3.20	3.06	0.22
	Day of AI1	3.00	3.10	3.30	3.11	0.22
	90 d post AI	3.10	3.20	3.40	3.23	0.21
Time period (ΔBCS)						
Primiparous						
	Calving to 21 DIM	-0.25	-0.20	-0.10	-0.160	0.14
	Calving to 56 DIM	-0.30	-0.20	-0.10	-0.220	0.18
	56 DIM to AI[3]	0.00	0.10	0.20	0.038	0.12
	AI to AI + 90 d[3]	0.00	0.10	0.20	0.090	0.13
Multiparous						
	Calving to 21 DIM	-0.30	-0.20	-0.10	-0.199	0.18
	Calving to 56 DIM	-0.48	-0.30	-0.20	-0.322	0.21
	56 DIM to AI[3]	0.00	0.10	0.10	0.053	0.15
	AI to AI + 90 d[3]	0.00	0.10	0.24	0.139	0.17
Overall						
	Calving to 21 DIM	-0.30	-0.20	-0.10	-0.182	0.16
	Calving to 56 DIM	-0.40	-0.30	-0.10	-0.277	0.20
	56 DIM to AI[3]	0.00	0.10	0.20	0.047	0.14
	AI to AI + 90 d[3]	0.00	0.10	0.20	0.120	0.16

Table 2.2: Least square means (SE) for BCS by pregnancy loss status in primiparous and multiparous cows at multiple time points. Covariables that remained in the full model included season of conception and DIM at AI category. Interactions tested were not significant and were removed from the models.

	Pregnancy loss[1]		
Time point (BCS)	No	Yes	P-value
Univariable model			
Primiparous			
Calving	3.36 (0.002)	3.34 (0.01)	0.26
21 DIM	3.20 (0.002)	3.18 (0.01)	0.02
56 DIM	3.14 (0.003)	3.11 (0.01)	0.002
Day of AI	3.18 (0.003)	3.17 (0.01)	0.24
90 d post AI	3.27 (0.003)	3.26 (0.01)	0.24
Multiparous			
Calving	3.31 (0.003)	3.31 (0.009)	0.81
21 DIM	3.12 (0.003)	3.11 (0.008)	0.18
56 DIM	2.99 (0.003)	2.98 (0.009)	0.31
Day of AI	3.05 (0.004)	3.05 (0.009)	0.47
90 d post AI	3.20 (0.004)	3.17 (0.010)	0.006
Full model			
Primiparous			
Calving	3.36 (0.003)	3.35 (0.01)	0.28
21 DIM	3.20 (0.003)	3.18 (0.01)	0.06
56 DIM	3.13 (0.003)	3.10 (0.01)	0.009
Day of AI	3.20 (0.003)	3.18 (0.01)	0.09
90 d post AI	3.28(0.003)	3.26 (0.01)	0.20
Multiparous			
Calving	3.31 (0.003)	3.31 (0.009)	0.31
21 DIM	3.11 (0.003)	3.09 (0.009)	0.11
56 DIM	2.97 (0.003)	3.96 (0.009)	0.21
Day of AI	3.06 (0.003)	3.04 (0.009)	0.07
90 d post AI	3.23 (0.004)	3.19 (0.01)	0.0005

[1] Cows were diagnosed pregnant via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 of gestation.

Table 2.3: Least square means (SE) for ΔBCS by pregnancy loss status in primiparous and multiparous cows at multiple time points. Covariables that remained in the full model included season of conception and DIM at AI category. Interactions tested were not significant and were removed from the models.

	Pregnancy loss[1]		
Time period (ΔBCS)	No	Yes	P-value
Univariable model			
Primiparous			
Calving to 21 DIM	-0.16 (0.002)	-0.17 (0.009)	0.33
Calving to 56 DIM	-0.21 (0.003)	-0.24 (0.01)	0.06
56 DIM to AI	0.04 (0.002)	0.06 (0.008)	0.0014
AI to 90 d post AI	0.09 (0.002)	0.08 (0.009)	0.80
Multiparous			
Calving to 21 DIM	-0.19 (0.003)	-0.21 (0.008)	0.07
Calving to 56 DIM	-0.32 (0.003)	-0.33 (0.01)	0.11
56 DIM to AI	0.05 (0.002)	0.05 (0.007)	0.40
AI to 90 d post AI	0.14 (0.003)	0.11 (0.008)	0.0013
Full model			
Primiparous			
Calving to 21 DIM	-0.16 (0.003)	-0.17 (0.01)	0.48
Calving to 56 DIM	-0.23 (0.004)	-0.26 (0.01)	0.12
56 DIM to AI	0.05 (0.002)	0.07 (0.007)	0.07
AI to 90 d post AI	0.08 (0.002)	0.08 (0.009)	0.82
Multiparous			
Calving to 21 DIM	-0.20 (0.003)	-0.22 (0.009)	0.04
Calving to 56 DIM	-0.33 (0.003)	-0.36 (0.01)	0.06
56 DIM to AI	0.09 (0.002)	0.09 (0.006)	0.80
AI to 90 d post AI	0.16 (0.003)	0.13 (0.008)	0.0027

[1] Cows were diagnosed pregnant via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 of gestation.

Table 2.4: Adjusted odds ratios (OR) and 95% CI for pregnancy loss[1] by category of BCS at multiple time points. Models included parity category (primiparous; multiparous), season of conception, and DIM at AI category[2] as covariables. Interactions tested were not significant and were removed from the models.

Time point	Calving		21 DIM		56 DIM		Day of AI		AI to 90 d post AI	
BCS category[3]	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
High	Ref.[4]	-	Ref.	-	Ref.	-	Ref.	-		
Medium	1.05 (0.87-1.28)	0.61	1.09 (0.91-1.45)	0.34	1.17 (0.94-1.45)	0.16	1.19 (0.99-1.44)	0.06	1.17 (0.97-1.41)	0.11
Low	1.09 (0.89-1.34)	0.40	1.20 (0.99-1.45)	0.06	1.41 (1.12-1.79)	0.004	1.31(1.05-1.65)	0.016	1.38 (1.10-1.74)	0.006

[1] Cows were diagnosed pregnant via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 after AI

[2] Days in milk at AI were categorized as low (≤ 90 DIM); medium (91 to 150 DIM); and high (>150 DIM).

[3] Values for BCS at each time point were categorized using the quartile distribution as low (≤ lower quartile), moderate (interquartile range), and high (≥ upper quartile), separately for primiparous and multiparous cows.

[4] Ref. = reference

Table 2.5: Adjusted odds ratios (OR) and 95% CI for pregnancy loss[1] by category of ΔBCS at multiple time periods. Models included parity (primiparous; multiparous), season of conception, and DIM at AI category[2] as covariables. Interactions tested were not significant and removed from the models.

	Time period			
	Early lactation			
BCS change category	Calving to 21 DIM		Calving to 56 DIM	
	OR (95% CI)	p-value	OR (95% CI)	p-value
No loss[3]	Ref.[5]	-	Ref.	-
Moderate loss	1. 17 (0.97 – 1.47)	0.19	0.98 (0.75 – 1.28)	0.87
Large loss	1.46 (1.10 – 1.94)	0.009	1.30 (0.96 – 1.75)	0.08
	Proximity to AI			
BCS change category	56 DIM to AI[3]		AI to 90 d post AI[3]	
	OR (95% CI)	P-value	OR (95% CI)	p-value
No loss[4]	Ref.	-	Ref.	-
Loss	1.03 (0.85 – 1.24)	0.79	1.44 (1.15 – 1.81)	0.001

[1] Cows were diagnosed pregnant via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 of gestation.

[2] Days in milk at AI were categorized as low (≤ 90 DIM); medium (91 to 150 DIM); and high (>150 DIM).

[3] Large loss of BCS (top 25% of cows loosing BCS); moderate loss (bottom 75% of cows loosing BCS); or no loss (ΔBCS ≥ 0)

[4] Cows classified as no BCS loss or BCS loss.

[5] Ref. = reference

Table 2.6: Distribution of pregnancy loss[1] by disease categories in primiparous and multiparous cows at multiple periods. Models included parity (primiparous; multiparous), season of conception, and DIM at AI category[2] as covariables. Interactions tested were not significant and removed from the models.

	Primiparous Pregnancy loss			Multiparous Pregnancy loss			Overall Pregnancy loss		
	No	Yes	P-value	No	Yes	P-value	No	Yes	P-value
Disease ≤ 80 DIM									
Healthy	2,336 (94.9)	124 (5.04)	<0.001	2,411 (89.8)	275 (10.2)	<0.001	4,747 (92.2)	399 (7.75)	<0.001
Reproductive disease[3]	728 (88.7)	93 (11.3)		492 (85.0)	87 (15.0)		1,220 (87.1)	180 (12.9)	
Other disorder[4]	456 (94.0)	29 (5.98)		1,292 (86.9)	195 (13.1)		1,748 (88.6)	224 (11.4)	
Reproductive and other	237 (88.4)	31 (11.6)		540 (83.9)	104 (16.2)		777 (85.2)	135 (14.8)	
Disease from calving to 90 d post AI									
No	3,424 (94.1)	213 (5.86)	<0.001	4,123 (89.3)	494 (10.7)	<0.001	4,155 (93.5)	287 (6.46)	<0.001
Yes	333 (83.9)	64 (12.12)		612 (78.6)	167 (21.4)		4,337 (87.0)	651 (13.1)	
Disease from AI to 90 d post AI									
No	2,106 (95.9)	90 (4.10)	<0.001	2,049 (91.2)	197 (8.77)	<0.001	7,547 (91.4)	707 (8.57)	<0.001
Yes	16.51 (89.8)	187 (10.2)		2,686 (85.3)	464 (14.7)		945 (80.4)	231 (19.6)	

[1] Cows were diagnosed pregnant via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 of gestation.

[2] Days in milk at AI were categorized as low (≤ 90 DIM); medium (91 to 150 DIM); and high (>150 DIM).

[3] Reproductive disease included retained fetal membranes, metritis, and pyometra

[4] Other disorders included clinical hypocalcemia, clinical ketosis, left displaced abomasum, lameness, clinical mastitis, digestive problem, injury, and respiratory disease

Table 2.7: Adjusted odds ratios (95% CI) for pregnancy loss[1] by parity, season of conception, and category of disease diagnosed from calving to 80 DIM and from AI to 90 d post AI. Models included parity, season of conception, and DIM at AI category as covariables. Interaction tested were not significant and removed from the models.

Study variable		OR	95% CI	P-value
Parity category				
	Primiparous	-	-	
	Multiparous	1.71	1.47-1.99	<0.0001
Season of conception				
	Summer	-	-	
	Fall	1.16	0.92-1.45	0.20
	Winter	1.39	1.12-1.74	0.03
	Spring	1.17	0.93-1.48	0.17
DIM at AI				
	≤90	-	-	
	90 - 150 DIM	0.98	0.84-1.14	0.80
	>150 DIM	1.30	1.08-1.57	0.005
Disease ≤ 80 DIM				
	No disease	-	-	
	Reproductive disease[2]	1.86	1.53-2.24	<0.0001
	Other disease[3]	1.34	1.12-1.60	0.001
	Reproductive and other	1.83	1.48-2.27	<0.0001
Disease up to 90 d post AI				
	No	-	-	
	Yes	2.54	2.15-3.00	<0.0001
Disease from AI to 90 d post AI				
	No	-	-	
	Yes	2.03	1.75-2.35	<0.0001

[1] Cows were diagnosed pregnant via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 of gestation.

[2] Reproductive disease included retained fetal membranes, metritis, and pyometra

[3] Other disorders included clinical hypocalcemia, clinical ketosis, left displaced abomasum, lameness, clinical mastitis, digestive problem, injury, and respiratory disease

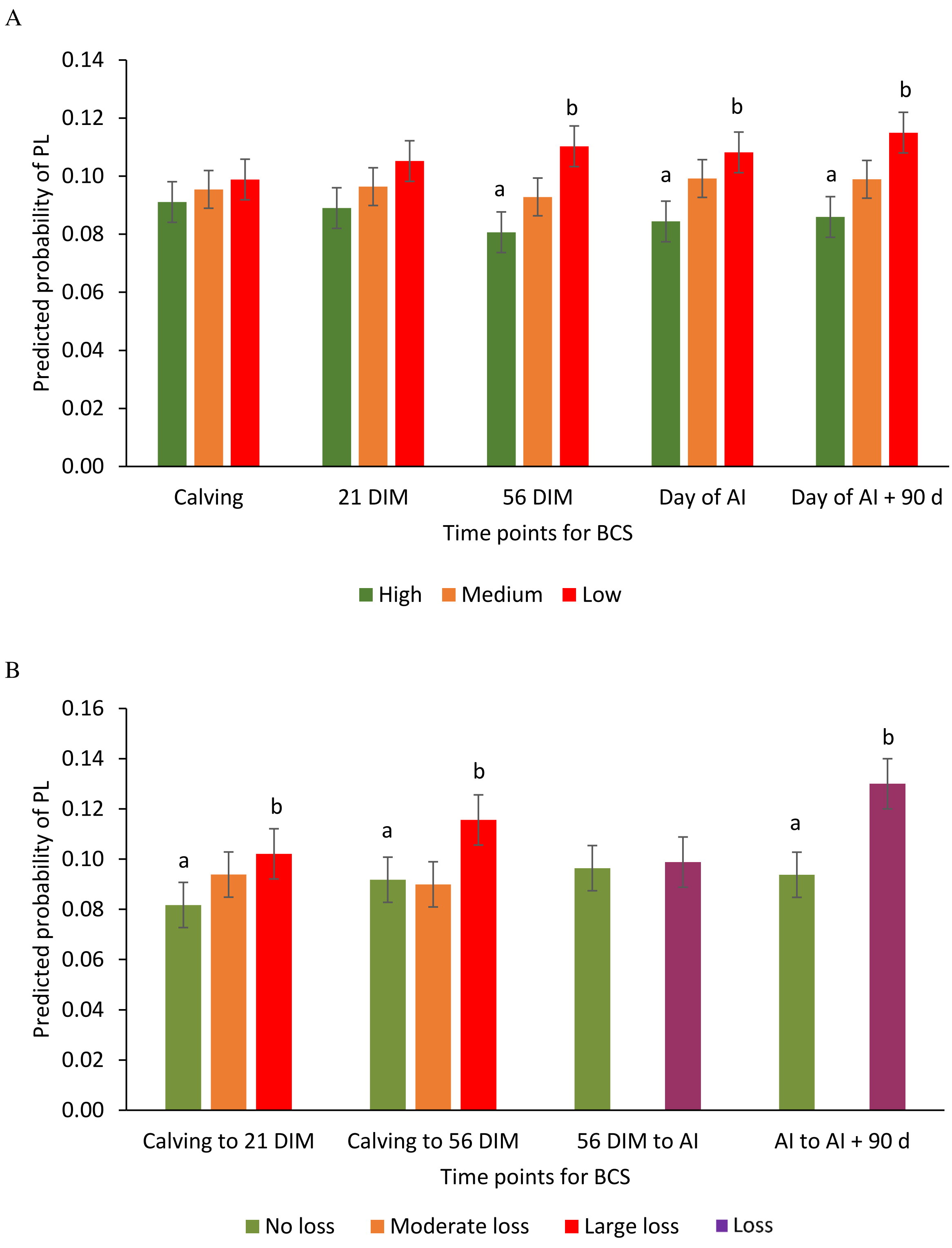


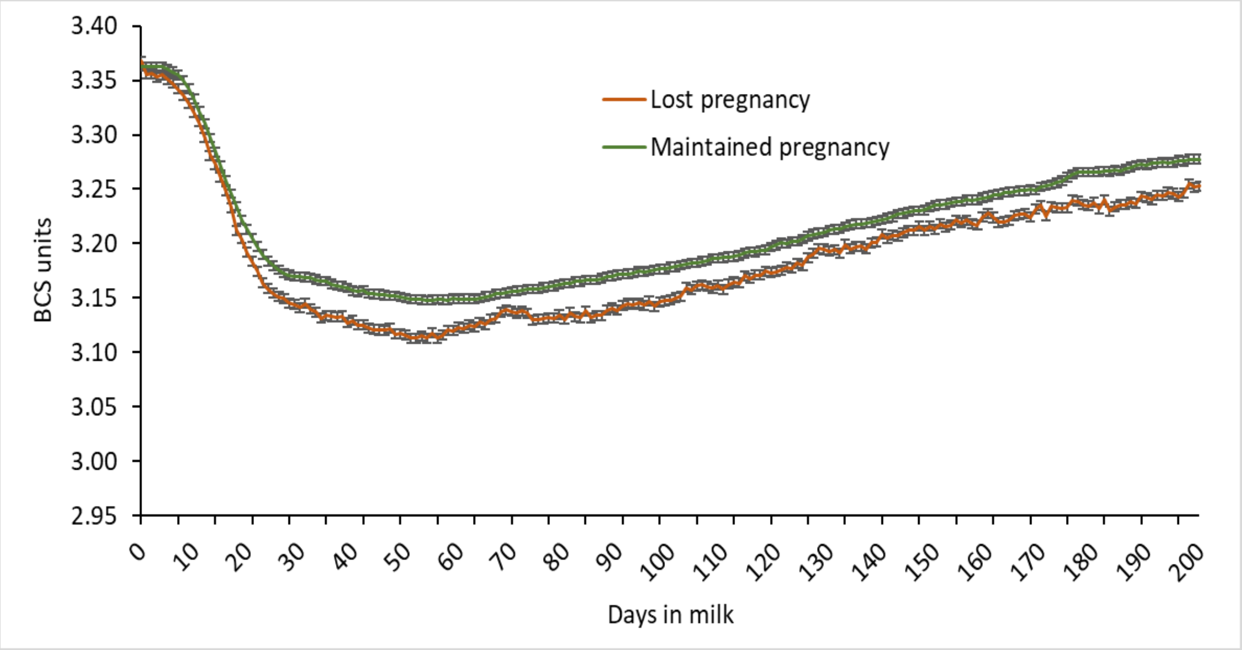
Figure 2.1: Predicted probabilities (LSM and SEM bars) for pregnancy loss (PL)[1] by BCS category (top panel, A) and BCS change category[2] (bottom panel, B).

[1] Cows were diagnosed pregnant via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 of gestation.

[2] BCS at each specific time point in primiparous cows (A) were categorized using the quartile distribution as low (≤ lower quartile), moderate (interquartile range), and high (≥ upper quartile). Changes in BCS (B) were categorized for Δ calving to 21 DIM and Δ calving to 21 DIM as large loss of BCS (LL; top 25% of cows loosing BCS); moderate loss (ML; bottom 75% of cows loosing BCS); or no change (NC; ΔBCS = 0).

For the periods Δ 56 DIM to AI and Δ AI to 90d post AI cows were classified as BCS loss or no change.

A: Primiparous



B: Multiparous

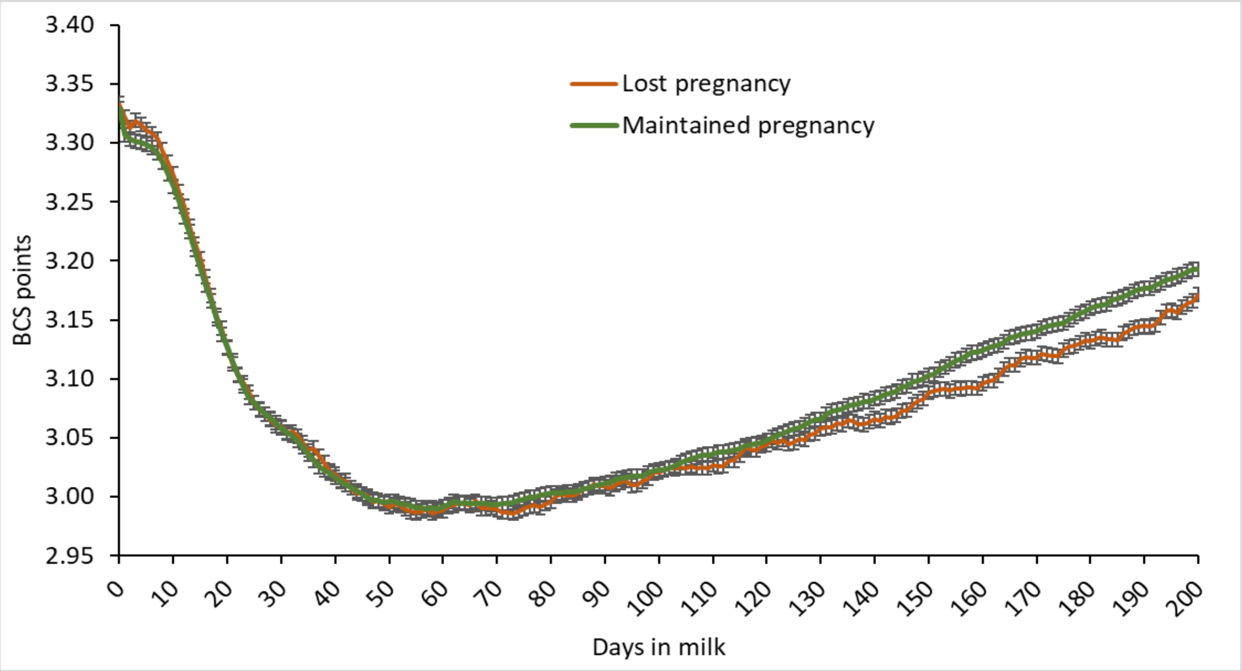


Figure 2.2: Dynamics of average (SEM) daily automated BCS during the first 200 DIM in cows that maintained (green line) or lost (orange line) their pregnancy[1] (A: primiparous; and B: multiparous cows). The blue arrows indicate the mean days in milk to AI of conception.
[1]Cows were diagnosed pregnant via transrectal ultrasonography on d 32±3 after AI and reconfirmed at d 80±3 of gestation.

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CHAPTER 3: GENERAL CONCLUSIONS

This thesis had the objective of characterizing the association between risk factors of pregnancy loss to improve health and pregnancy outcomes. The literature review included three main topics of transition period, reproductive efficiency, and reproductive challenges. The transition period is a critical time period within dairy production and plays a key role in the determination of the future pregnancy and lactation cycles a dairy cow will have. The literature review focused on the impacts of negative energy balance and immune function, and specifically how those can alter health and reproduction. Managing the transition period must take into account a multitude of risk factors including environment, management, milk yield, nutrition, and social dynamics. The transition period must be managed effectively as mismanaged nutrition can negatively alter immune function, which can lead to a decrease in reproductive efficiency.

Reproductive efficiency has been at the forefront of dairy research as advancements in this field can improve the sustainability of dairy production. The literature review presented here discusses advancements in dairy production including artificial insemination and synchronization. Artificial insemination (AI) has improved the genetic potential of dairy cows to use fewer less animals to produce higher quantities of milk. AI allows for the maintenance of elite sires with superior semen quality as well as the use of sexed semen. Embryo transfer and ovum pick up have also furthered genetic potential. Synchronization has allowed for the minimization of estrus detection use, leading to more precise service and increased pregnancy rates. Pregnancy detection has typically been transrectal palpation and has advance to the use of ultrasonography and blood testing for early pregnancy readings. The knowledge of early conception after breeding culminates knowledge of individual animal performance, and areas for improvement.

Considering the success of reproduction in the dairy industry, reproductive challenges are now more observable. It is a goal for farmers to achieve pregnancy after a single AI service and due to improved pregnancy detection, it is easier to monitor the presence of pregnancy after each service. The literature reviewed here focused on anovulation, conception rate at first artificial insemination, and pregnancy loss. Anovulation leads to decreased pregnancy rates as the lack of cyclicity prolongs time of insemination. Additionally, animals that experience anovulation have a higher likelihood of pregnancy loss.

This thesis research evaluated the presence of pregnancy and subsequent loss to determine risk factors. Although pregnancy loss can occur throughout pregnancy, the rates of loss are significantly higher earlier in pregnancy. Not only are there negative health effects following pregnancy loss, but additionally economic impacts culminated from reduced future reproductive performance, and reduced milk yield. Environmental effects are often centered on heat stress in warmer seasons. Management factors, however, can greatly alter pregnancy especially the nutritional management and its influence on physiological state. This thesis discussed the effects of body condition score on pregnancy loss and the potential to monitor using automated scoring aids to score animals more frequently. Additionally, it is known that the presence of disease will negatively affect pregnancy. Since health events and body condition are such critical risk factors of early pregnancy loss, there was an opportunity to investigate these factors in chapter 2 of this thesis.

The objective of chapter 2 was to investigate the association between body condition, change in body condition, and disease on pregnancy loss in Holstein cows. This study utilized an automated camera system 32d after AI to determine BCS and diagnose pregnancy via transrectal ultrasonography. Subsequently, animals were reconfirmed as pregnant or not pregnant at d 80 after AI. The study population utilized animals that were pregnant at the first determination of pregnancy and were subsequently open at the second transrectal ultrasound intended to confirm pregnancy. Chapter 2 found low BCS and loss of BCS to be associated with pregnancy loss, specifically when occurring close to AI. Additionally, disease had a negative impact on pregnancy in Holstein cows.

Pregnancy loss is multifactorial, and importantly both body condition and disease can greatly increase the risk of pregnancy loss. The study in this thesis provides beneficial knowledge in understanding major risk factors of pregnancy loss and categorizing the importance of these risk factors. The continuation of automated body condition scoring technology can improve both the ability to predict reproductive performance, and aid prevention of negative health outcomes.

Overall, there is still opportunity for improvement of reproductive outcomes in dairy cows and although there have been remarkable advancements in dairy reproduction, there are still difficulties that need to be addressed in future studies.